Vol. 17 No. 2

PLANT PHYSIOLOGY

APRIL, 1942

"VEGETABLE DYNAMICKS" AND PLANT TISSUE CULTURES[1]

PHILIP R. WHITE

(WITH TEN FIGURES)

Some time ago, at a dinner at the Yale Medical School, I was asked to occupy the chair specially built for the Honorable WILLIAM HOWARD TAFT when he was Professor of Jurisprudence at Yale. Mr. TAFT had weighed well over three hundred pounds at that time so that I was made to feel very, very small thereby. Now you have done me the honor to link my name, however tenuously, with that of the Father of our discipline, STEPHEN HALES. It is hard to know whether to be inflated by the connection or deflated by the comparison. Whatever *should* be my reaction, I deeply appreciate the honor. In accepting that honor, I shall try not to impose upon your patience by talking to you too long.

STEPHEN HALES' interests, as a minister of the gospel and a humanitarian, led him to touch upon anything which could contribute to the welfare of his fellow men and redound to the glory of Almighty God. The processes by which God's creatures function in their everyday lives could, according to his lights, only show the perfection of the works of the Great Planner. HALES set to work to discover what details he could in that Plan, in a spirit of humility. And, since plants were also God's creatures and HALES was a good gardener as well as a good shepherd, he turned much of his interest to a study of what he chose to call "Vegetable Staticks."

In HALES' day, the classification of plant parts into tissues, proposed by the Greeks, and especially by THEOPHRASTUS, had been for the most part forgotten, and his considerations were, wherever possible, of the plant as a whole. But the philosophical analyses of THEOPHRASTUS and the anatomical work of GREW and MALPIGHI, HOOKE, and LEEUWENHOEK had already laid the foundations for another point of view. As our knowledge of vital functions increased, so did the complexity of the plan revealed increase, until it became an almost hopeless task to unravel the details while trying to encom-

[1] The seventh STEPHEN HALES address, read before the American Society of Plant Physiologists at Dallas, December 29, 1941.

pass the whole picture, even as regards a single creature. Thus it happened that, about a century after HALES' epoch-making work, there came to be formulated the so-called "Cell Theory" which said, in essence, "It is not necessary to encompass the plan of a plant or animal in its entirety and all at once, for each creature is made up of a sum of many smaller, simpler,

FIG. 1. STEPHEN HALES, D.D., F.R.S., at the age of 81. (From the portrait by THOMAS HUDSON in the National Portrait Gallery, London.)

potentially autonomous organisms, the cells. Learn to understand the plan of a single cell. From that the greater Plan can be built up." That theory, while sharing the field with the older concept of the organism as a whole, has nevertheless dominated much of our thought as cytologists, as anatomists, as geneticists, and as physiologists for the past century.

HALES developed a series of techniques for studying the organism as a whole which are still useful. But, as we have passed from the larger aspects to smaller and still smaller ones, the technical difficulties have increased. To deal with single cells, we must have methods adapted to that end. HABERLANDT saw that clearly forty years ago and set in train the series of explorations of which I am but one of the momentary advance guard.

We cannot yet grow single somatic cells of higher plants, as HABERLANDT would have had us do. Some day I am certain someone will be able to do so. But we can grow comparatively simple organs and tissue complexes. A few of you, I know, have followed the steps by which that has been accomplished. Others I am sure have not, and I hope I will be forgiven if I review briefly for their benefit the development of the field. The aim was, as we have said, the study of single somatic cells of higher plants. The method aimed at was the cultivation of those cells, their study as separate entities, and their subsequent aggregation into more and more complex groupings until full-blown organisms could again be attained. In a series of investigations, HABERLANDT attempted to grow single green somatic cells, unsuccessfully, as did many of his students. One of those students, KOTTE, and ROBBINS in this country in 1922 compromised by using, not cells, but organs—root tips—with some success. But the results were not sufficiently promising to encourage further work and the attempt was not pushed further. Somewhat later the problem came to my attention and I decided, on theoretical grounds, that in the development of the method the first study should be made on three types of materials—embryos, seed primordia, and root tips. Of these, the last proved most satisfactory. With them a beginning has been made toward a solution of the problems involved.

Fundamental, of course, was the need of an environment that should replace all the *essential* features of that in which the cell or tissue or organ finds itself in the organism, while discarding the nonessentials—as regards physical and chemical composition of that environment. Temperature, light, oxygen tension, acidity, physical state, motion, conditions of contact, as well as ionic composition, content in energy sources, hormonal and vitamin content, and similar aspects, all had to be studied. And, while many of these requirements were likely to prove similar for all cells, others might not prove similar. We have solved all of these problems in a preliminary way. Those solutions are in many cases obviously not yet the most perfect ones possible and the work still goes on.

In spite of the complexities of the problem, it is already clear that we *can* grow excised plant organs and tissues *in vitro* and maintain fairly "normal" behavior. A strain of roots of tomato has been under continuous cultivation *in vitro* for more than nine years and has been used in hundreds of experiments during that period. The average growth rate over the entire

period has been about 6 mm. per culture per day. Occasionally a single root may grow 50 mm. or more in a single 24-hour period, but such rates are not common. Roots of many other species of plants have been grown in similar manner (fig. 2).

As was to be expected, not all roots grow equally well in any given environment, but we now are able to grow not only these but embryos and undifferentiated tissue masses from many species—Salix, Helianthus, Ulmus,

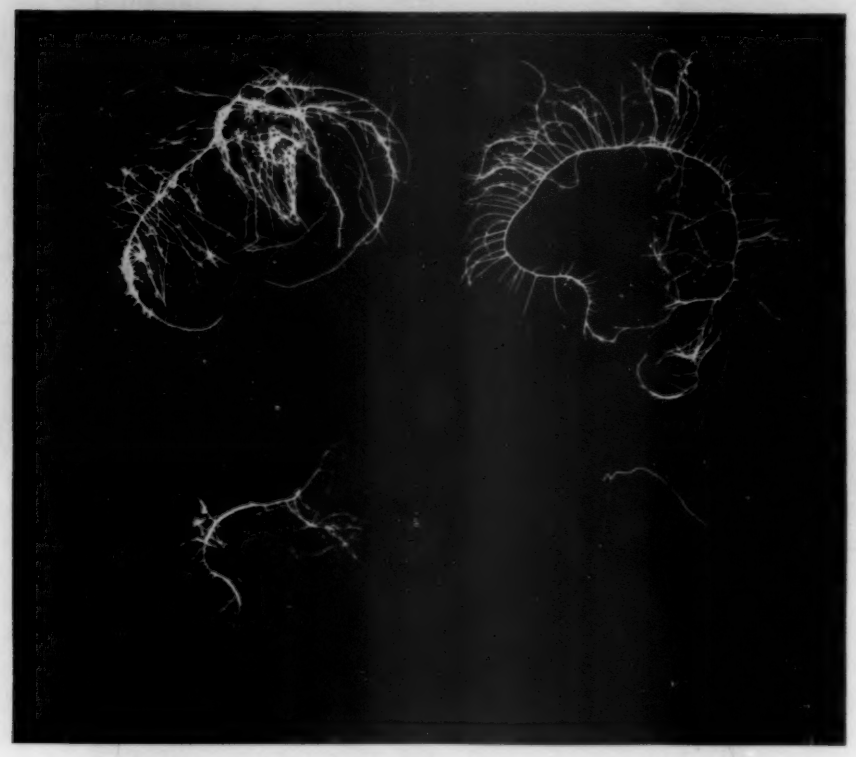


FIG. 2. Excised roots of *Petunia violacea* (upper left), *Trifolium repens* (upper right), *Nicotiana langsdorffii* (lower left), and *Daucus carota* (lower right), established for ten passages or more *in vitro*. The pictures represent the growth in three weeks from pieces about 15 mm. in length. ×0.5. (Photograph by J. A. CARLILE.)

Prunus, and others—for long periods and to control the environments in which they grow with a considerable degree of precision. The *technique* for which HABERLANDT was searching is thus now available, and it is, I trust, because of the part I have played in developing that technique, rather than for any particular application, that you have honored me this evening.

It would be superfluous for me to review our findings in detail here. It is my task, rather, to point out the *directions* in which we have already

moved and perhaps to outline briefly some of the new fields in which we may hope to find opportunities for further work.

As I have already noted, problems in nutrition were among the first to present themselves, and the way in which they have been solved points to other possibilities in the same direction. A nutrient adequate for the support of "normal" growth has been developed. If we omit any constituent of that nutrient, with the exception of sodium and chlorine, there results a

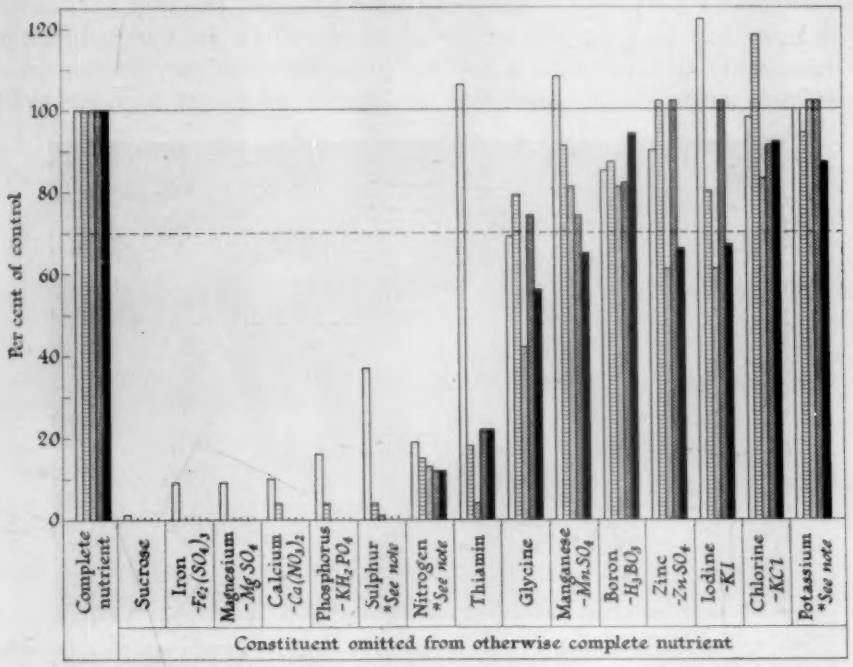


FIG. 3. Histogram showing the effects on growth of excised tomato roots of omitting each of 15 nutrient constituents from an otherwise complete nutrient. Each column represents the average linear increase of 20 or more roots over a week's period in the particular nutrient, expressed as percentage of the increase during the same period of 20 roots grown in a complete nutrient. The five columns in each group represents five consecutive passages. "Minus sulphur" should read "minus sulphate," since a trace of sulphur was present in the thiamin used. "Minus nitrogen" should likewise read "minus nitrate," since a trace of nitrogen was present in the glycine used. Although no deficiency in *linear* increase occurs in the absence of potassium, there is a very marked decrease in branching and in the diameter of the roots. Results differing from the control by less than 30 per cent. (above the horizontal dotted line) are for any given passage not of certain significance, although the consistent trends in the "minus manganese" and "minus boron" groups lend a probable significance to the results although they fall numerically within this range.

more or less rapid degeneration in the growth behavior. With the omission of sugar, magnesium, calcium, phosphorus, sulphate, nitrate, and iron, this

degeneration is catastrophic. With thiamin, iodine, and manganese, it is progressive but pronounced, while with glycine, boron, zinc, and potassium it is slow and more qualitative than quantitative. Some individual points here are worth emphasizing. Why, in a chlorophyll-free tissue, should omission of iron have such a catastrophic effect? Iron is not tolerated at an ionic concentration above about 2×10^{-5} (=2/100,000) M, yet its omission results in an abrupt and complete, but reversible, cessation of growth. That its function is an indispensable one is evident. What that function is we do not yet know, but the tissue culture technique offers us a hope of finding out. Omission of iodine results in a slow but progressive tendency for the apical meristem to lose its dominance over the laterals, producing a progressively

FIG. 4. Effects on growth of excised tomato roots of nutrients containing (reading left to right) 0.00, 0.06, 0.20, 0.60, 2.00, and 6.00×10^{-5} M $Fe_2(SO_4)_3$. No growth occurs in either the lowest or the highest concentration. ×0.5. (Photograph by J. A. CARLILE.)

shorter and bushier type of growth without any significant diminution in amount of tissue laid down. Omission of potassium has just the opposite effect, the apex elongating at a rate equal to that in the presence of potassium but becoming progressively more and more slender and with fewer and fewer branches. These morphogenetic effects need to be studied in more detail. They remind one of the recently published correlations between zinc, boron, and auxin activities.

Nor are the problems restricted to the inorganic constituents of the nutrient. Omission of sucrose results in cessation of growth. How much of this is to be attributed to the removal of a source of energy and how much to a disturbance in the osmotic relations involved? If we maintain the osmotic relations by use of a relatively inert inorganic substitute such as NaCl and

then vary the energy relations, we find that the latter are of paramount importance. The use of sucrose has another interesting aspect. Sucrose is a classic plasmolytic agent which is said not to penetrate the cell membranes. Yet here we find its major function to be in the interior of the cell,

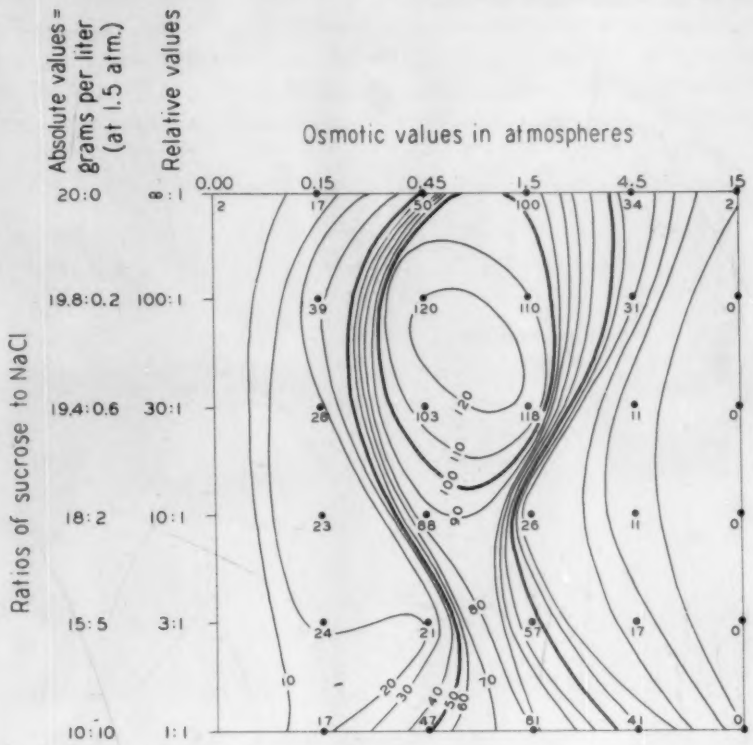


FIG. 5. Isopleth diagram showing the effects on growth of excised tomato roots of varying the ratios of sucrose (a nutrient *and* osmotic agent) and sodium chloride (an osmotic agent but *not* a nutrient) at five different total osmotic values in addition to their complete omission. All variants were studied in the presence of an otherwise complete and balanced nutrient. Osmotic values above about two atmospheres or below about 0.3 atmosphere were unsatisfactory with all combinations of sucrose and sodium chloride. All combinations in which NaCl represented more than one-tenth by weight of osmotically active substances or more than about six-tenths of the osmotic value were likewise unsatisfactory. The best results were obtained with little or no NaCl and an osmotic value around one atmosphere. (The osmotic value in most animal tissue culture nutrients is maintained almost entirely by use of NaCl.)

not on the outside. And it cannot be satisfactorily replaced by either dextrose or fructose, which are said to penetrate to the interior of the cell easily. When I published results showing this preference for sucrose rather than dextrose, I let myself in for some criticism from those who followed

the textbook story. But I also received a very interesting and heartening letter from Dr. ANNIE MAY HURD-KARRER, saying that in 1926 she had been puzzled by evidence of a similar preference by Ustilago infecting corn. Perhaps the question needs to be re-examined. The textbooks have been known to be occasionally wrong.

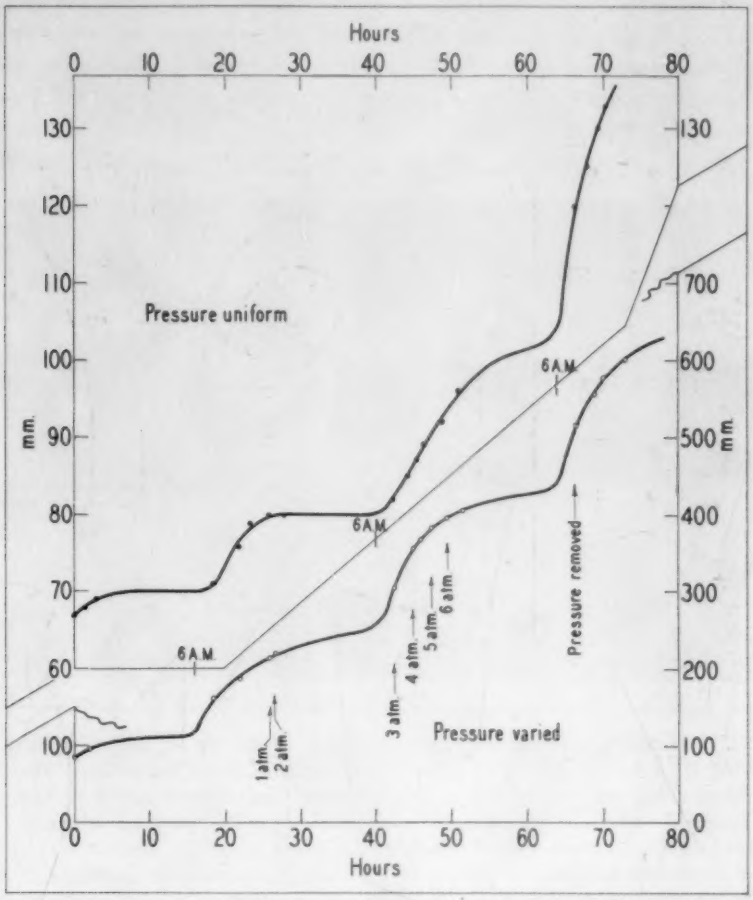


FIG. 6. Two curves showing the rates at which single growing isolated tomato roots secrete fluid into a manometer when no external pressure is imposed to retard the flow (above) and when a graded series of opposing pressures is imposed (below). (Amer. Jour. Bot. 25: 226. 1938.)

The other organic constituents of the nutrient have also presented interesting problems. KOTTE had used meat extract as a source of organic materials, while ROBBINS had used a yeast extract. Yeast can be fractionated into significant parts by various methods. Fractionation with 85 per cent. and 100 per cent. alcohol proved to be a valuable procedure and led us

ultimately to replace the yeast with thiamin, soluble in 100 per cent. alcohol, and glycine, soluble in 85 per cent. but insoluble in 100 per cent. alcohol. Concentration curves similar to that already mentioned for iron can likewise be obtained with glycine.

These are but isolated questions. How important is auxin in the economy of an excised member? What is the relation between pyridoxine and protein synthesis in the plant? How does oxygen tension affect morphogenetic patterns? Can histological behavior under deficiency conditions give us clues to the real functions of any of these materials? These are

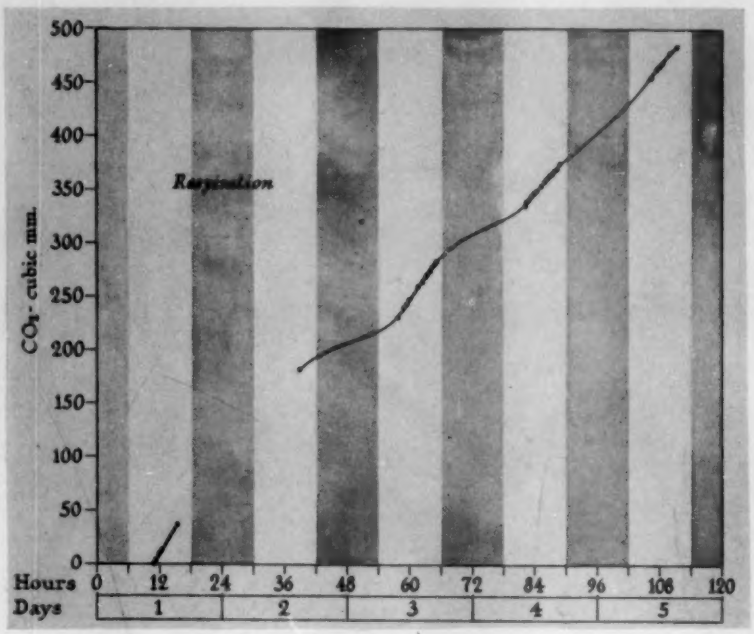


FIG. 7. The respiration rates of similar excised roots over a corresponding period, showing the same day-night rhythm in respiration as is shown in fluid secretion.

but a few characteristic nutritional problems that have been or can be attacked by means of a tissue culture technique.

But the value of the method is by no means limited to nutritional problems. We were from the start interested in using it in dealing with a variety of problems in plant pathology. Some of these have led us into unexpected paths. The method by which viruses travel through the host has long been a puzzle to phytopathologists. This is paralleled and perhaps intimately bound up with the methods by which foods move in the plant. And it is also conceivably bound up with the methods by which water may sometimes be moved through the plant when certain external forces are temporarily

in abeyance. The picture of water movement as a function of vital activity of plant tissues has long been a puzzling one. Five years ago, I set out to study this picture as exemplified in growing excised roots, where the complicating factors of photosynthesis, evaporation, trunk expansion and contraction under temperature influences, local hydrolysis, etc. could be eliminated. The results have proven of unexpected interest. By attaching special capillary manometers to single growing excised roots, we have been able to demonstrate a continuous, rhythmic flow of liquid from root tip to

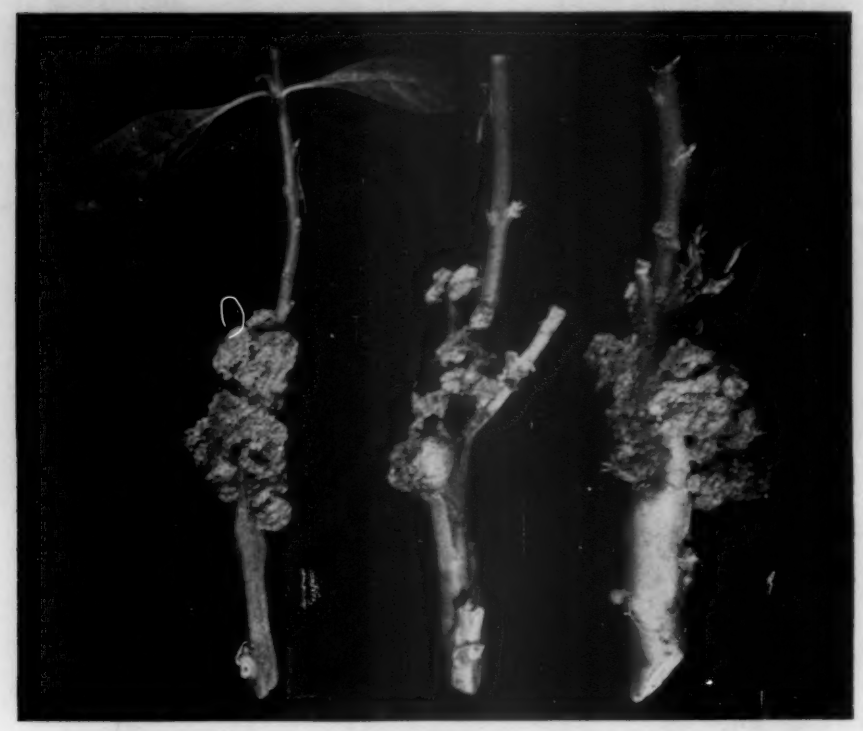


FIG. 8. Typical spontaneous galls on plants of the hybrid, *Nicotiana langsdorffii* × *N. glauca*, similar to those which have served as material for *in vitro* cultures of undifferentiated tissue masses. These galls are of genetic origin and are not transmissible. ×0.2 (Photograph by J. A. CARLILE.)

base and out through the cut surface. This flow can be shown to develop extraordinary hydrostatic pressures quite sufficient to raise water to the tops of tall trees. And its behavior is such as to indicate a dependence on respiratory processes. We have not solved the problem as far as demonstrating a detailed mechanism is concerned. But we have been able to establish in an unexpected and striking way certain significant facts which tend to support and enlarge upon STEPHEN HALES' old concept of water

secretion as a vital function of root tissues. Those results at the time they were first reported seemed again to run counter to accepted textbook theories. But they have since found support in work of ROSENE, VAN OVERBEEK, GROSSENBACHER, MASON and PHILLIS, and others. And the picture which MASON and PHILLIS draw certainly corresponds rather closely to that of the contractile vacuole in protozoa, which is another little-understood phenomenon.

Recently we have come back to one of the first problems for the solution of which the value of a tissue culture technique was foreseen, that of the etiology of neoplasia. Making use of the nutrients and methods developed

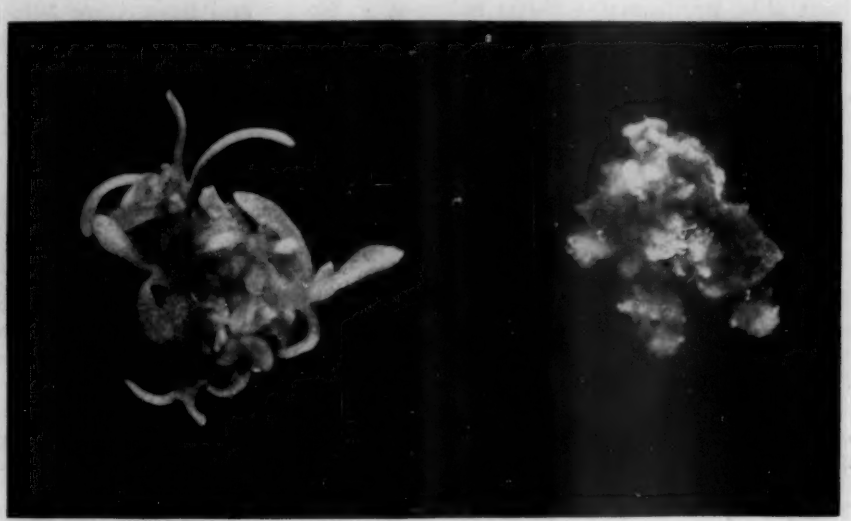


FIG. 9. Differentiation of gall tissue cultivated *in vitro* on a semi-solid medium (right) and in a chemically similar but physically dissimilar (liquid) medium (left). It is believed that the differences in behavior are the result of differences in oxygen supply in the two nutrients. ×4. (Bull. Torrey Bot. Club **66**: 509. 1939.)

in the study of excised roots, we first studied a type of neoplastic growth which develops spontaneously in certain Nicotiana hybrids as a result of some sort of chromosomal upset. Plants of these hybrids start out as normal-appearing individuals, but wherever any injury such as the abscission of a leaf or the breaking of a flower stalk occurs, a tumor-like callus forms. These tumors gradually spread over the entire plant and it ultimately becomes entirely tumorous and dies. Cultures can be made from the sterile interiors of these tumors and grown *in vitro* indefinitely. Here they can be subjected to all sorts of experimental procedures. One of the most striking results is obtained by varying the oxygen tension, by simply raising the cultures on the surface of or immersing them in the nutrient. Under the former conditions, the cultures grow as undifferentiated masses showing

evidence of only a very limited capacity for morphogenetic differentiation. Under the latter conditions, on the other hand, they undergo a profound metamorphosis, producing leaves, stems, and in some cases roots. The result presents striking evidence of the way in which long latent characteristics can be abruptly evoked by relatively simple means.

Having learned to handle a spontaneous neoplasm, the step to one of biologic or parasitic origin was a logical one. This we have made in studying certain types of crown-gall tumors, the bacteria-free metastatic tumors which occur in certain species of composites. And here we have again come upon unexpected and, we believe, very important phenomena, for we have been able, by means of tissue cultures, to isolate and grow these tumor tissues; to subject them to all sorts of bacteriological and serological tests as

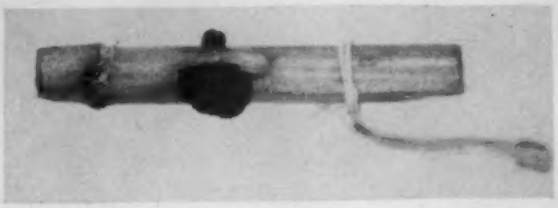


FIG. 10. A gall on a sunflower plant experimentally produced by implanting at the cambium level a fragment of tissue derived from a bacteria-free tumor arising on a plant inoculated with *Phytomonas tumefaciens*. The tissue implanted was isolated, grown *in vitro* for ten passages, tested repeatedly to establish its freedom from bacteria, and then used as "inoculum" to produce this gall. Galls of this type are transmissible from one plant to another, but their origin is not yet fully understood. ×1. (Photograph by J. A. CARLILE.)

well as altered nutritional environments; to demonstrate unequivocally their freedom from the original inciting agent; and then, by grafting them not only into the original "host" but to other hosts as well, to demonstrate the fundamental changes which they have undergone in acquiring a permanent and self-propagating neoplastic character. We believe that we will be able to follow step by step the development of this character, the etiology of neoplasia. And we have hopes of later being able to proceed still another step to a study of neoplasia of controllable physiological origin.

These are but a few examples to indicate the proven as well as potential scope of the field which the technique of tissue cultures opens to us. STEPHEN HALES was not thinking in terms of this sort. But these problems are such that their solution, by whatever means, can give us greater insight into the workings of biological entities, cells, tissues, organs, and thereby of the organisms which were HALES' field of interest. They may well help us to understand some new bits of the universal plan which he sought to elucidate.

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

COMPOSITION OF ALCOHOLIC LEAF EXTRACT AND THE ENTIRE LEAF OF IRISH POTATOES AS INDICES OF SOIL FERTILITY

C. E. Beauchamp

In a previous paper the writer reported investigations on the composition of dried alcoholic extract of potato leaves taken from plots which had received different fertilizer treatments (**1**). In this investigation it was found that a very pronounced relationship existed between the fertilizers applied and the nitrogen, phosphorus, and potash present in the alcoholic extract of the leaves; between potash and the yield; and between the fertilizers and the yield.

In the present paper the writer endeavors to compare the composition of the dried alcoholic extract of the leaves of Irish Cobbler potatoes with that of the entire leaves of the same crop to determine which of these bodies is the better indicator of soil deficiencies. In both cases the magnitude, intensity of nutrition, and N–P–K units as described by Thomas (**5, 7**) were used as an aid in the interpretation of quantitative and qualitative relationships of nutrition. Details regarding the field experiments, leaf sampling, alcoholic extraction, and methods of analysis may be found in a previous paper (**1**).

Results

Alcoholic extracts

In this earlier work the composition of the dried alcoholic leaf extract from the various plots was correlated with the respective yields. Since, however, the present study presents a comparison of the contents of dried alcoholic leaf extracts with those of the entire leaves, essential data from the previous paper will be presented in the development of this study.

The alcoholic extract of the leaves comprises most of the leaf pigments as well as phospholipoids. Most phosphates excepting dipotassium hydrogen phosphate and the salts of potash, calcium, and magnesium appear to be very little soluble in alcohol. Consequently, most of the phosphorus, potash, calcium, and magnesium removed by the alcoholic extraction comprises the water-soluble elements present in the sap of the green leaves taken for analysis and that dissolved in the 4 per cent. of water present in the 96 per cent. alcohol used. Although dipotassium hydrogen phosphate is very soluble in alcohol, it does not follow that the potash and phosphorus removed by the extraction correspond entirely to the amount of these elements combined as such, since their ratio does not conform to that in which they are present in this compound.

From the foregoing, it is very likely that the content of plant-food elements of the alcoholic leaf extract represents the major part of those elements present in the cell sap of the leaves which are immediately available for the synthetic processes of the foliage.

Table I shows a very pronounced relationship between the fertilizers applied, the composition of the dried alcoholic leaf extract, and the yield. The lowest production of tubers per acre occurred in the case of the check treatment. This, in turn, corresponds to the smallest total content of nitrogen, phosphorus, and potash in the dried alcoholic leaf extract. The next lowest-yielding plots after the checks, are those receiving the incomplete minus-potash fertilizer mixtures nos. 3, and 6. The dried alcoholic leaf extracts of these treatments are considerably higher in both nitrogen and potash and only slightly higher in phosphorus than the alcoholic extract of the check treatment. On the other hand, complete fertilizer treatments nos. 2, 4, 5, 7, and 8 were the highest in yields and also in the total percentage of the fertilizing elements found in their respective alcoholic leaf extracts.

TABLE I

COMPOSITION OF THE ALCOHOLIC LEAF EXTRACT AND CROP YIELDS

TREATMENTS	YIELD PER ACRE	NITROGEN N	PHOSPHORUS P_2O_5	POTASH K_2O	$N+P_2O_5+K_2O$	K_2O/P_2O_5
	cwt.	%	%	%	%	
(1) Check	17.39	0.429	0.155	2.87	3.454	18.5
(2) 6–8–10	105.28	0.974	0.480	9.77	11.224	20.3
(3) 8–10–0	75.67	1.034	0.233	3.97	5.237	17.0
(4) 8–10–10	103.63	1.042	0.534	10.76	12.336	20.1
(5) 8–10–15	112.80	1.027	0.509	11.29	12.826	22.2
(6) 10–8–0	79.43	1.306	0.237	5.84	7.383	24.6
(7) 10–8–10	105.98	1.050	0.515	11.27	12.835	21.8
(8) 10–8–15	120.79	1.208	0.568	9.97	11.746	17.5

No significant difference is apparent when the nitrogen contents of complete and incomplete (minus-potash) treatments are compared. This is only logical since both of these treatments furnished more than the minimum amount of nitrogen inducing a positive field response. It is very interesting to observe that the alcoholic leaf extracts from the complete fertilizer treatments are significantly higher in both phosphorus and potash than in the case of the incomplete (minus-potash) plots. From the standpoint of potash alone, this result would be expected since this element was withheld from the incomplete treatments. In the case of phosphorus the situation is different since this element was applied at practically the same rate in both complete and incomplete treatments. These results indicate an apparent mutual relationship between phosphorus and potash in the synthetic processes of the leaves. This assumption is substantiated by the fact that in spite of

marked differences between the various treatments applied, the actual $K_2O : P_2O_5$ ratio calculated from the respective percentages of these elements in the alcoholic leaf extracts, seems to be fairly constant. These ratios are also shown in table I.

Composition of the leaf skeleton

The leaf skeleton represents in this study that portion of the leaves insoluble in alcohol. It contains, however, additional quantities of water soluble elements which could not be extracted with alcohol. Lazo and Bonazzi (**4**), studying the localization of phosphorus in the sugar cane plant, found phosphorus in the leaves after removing the chlorophyll of the latter by means of alcohol and ether. Potato leaf samples were macerated with different water-alcohol mixtures and it was found that the extraction of nitrogen, phosphorus, potash, lime, and magnesia increased as the ratio of water to alcohol was raised. Consequently, the leaf skeleton in the present case contains a considerable quantity of water-soluble nutrients that were not removed in the alcoholic extraction. Its contents of nitrogen, phosphorus, and potash appear in table II.

The nitrogen, phosphorus, and potash content of the leaf skeletons as shown in this table, when correlated with the tuber yield per acre, do not show the sharp contrasts obtaining in the case of the alcoholic leaf extract. There is a slight indication that an increment of nitrogen in the soil resulted in a small increase of this nutrient in the leaf skeleton. This is evident when the nitrogen content of the check treatment is compared with that of

TABLE II

Percentage of nitrogen, phosphorus, and potash in the leaf skeleton (Alcohol-insoluble leaf portion)

Treatments	Yield per acre	Nitrogen N	Phosphorus P_2O_5	Potash K_2O	Total
	cwt.	%	%	%	%
(1) Check	17.39	2.59	0.25	1.92	4.76
(2) 6–8–10	105.28	2.92	0.59	1.56	5.07
(3) 8–10–0	75.67	3.14	1.00	0.92	5 06
(4) 8–10–10	103.63	3.34	0.68	1.07	5.09
(5) 8–10–15	112.80	3.32	0.65	1.02	4.99
(6) 10–8–0	79.43	3.27	0.92	0.75	4.94
(7) 10–8–10	105.98	3.41	0.73	1.50	5 64
(8) 10–8–15	120.79	3.33	0.64	1.69	5.66

plots receiving 6 and 8 per cent. of this element in the fertilizer. Nevertheless, there does not seem to be any correlation between nitrogen in the leaf skeleton and the yield, since incomplete treatments show as much nitrogen in the leaf skeleton as complete treatments. This point, however, is in agreement with the corresponding finding in the alcoholic leaf extract. Ni-

trogen absorption does not seem to be affected by the phosphorus or potash intake but its efficiency in plant nutrition depends upon the proper equilibrium between the elements involved.

The phosphorus content of the leaf skeleton is noteworthy. The check treatment appears to have the lowest phosphorus content. The fact that the alcoholic extract of this treatment also showed the lowest phosphorus content indicates that the soil in question was deficient in this element. All complete fertilizer treatments showed a higher phosphorus content than did the check treatment. Of all fertilizer treatments under comparison, however, the incomplete ones, without potash, show the highest phosphorus content. This is entirely opposite to results in the corresponding case of the alcoholic leaf extract. It was pointed out that phosphorus appears to be present in the alcoholic extract in a more or less definite ratio with respect to the quantity of potash present. Consequently, the minus-potash treatment resulted in a low-potash, low-phosphorus content in its alcoholic leaf extract. Since this incomplete fertilizer treatment, however, received as much phosphorus as did the complete treatments, that portion of this nutrient not present in the alcoholic leaf extract was found in the leaf skeleton.

The contents of potash in the leaf skeleton from the various treatments is also noteworthy. The highest percentage of this element appears on the check treatment while the lowest figures was found on the minus-potash treatments. The content of this nutrient in the complete fertilizer treatments appears to be intermediate between these two. The figures which at first appear somewhat puzzling may be interpreted by assuming that the amount of potash present in the leaf skeleton is that portion of the total potash in the leaves which was not localized in the cell sap to function in the synthetic processes of the leaf.

Since the leaf skeleton in this study contains additional quantities of water-soluble nutrients that could not be removed by the alcoholic extraction, it may be interesting to calculate the total amount of nitrogen, phosphorus, and potash in the leaves in terms of the alcoholic extract in order to compare those values with the field response of the crop. Such figures appear in the following table III.

The total individual content of nitrogen, phosphorus, and potash in the leaves in terms of the dried alcoholic extract are practically lower in the check plots than in any of the fertilizer treatments applied. The only exceptions appear in the case of the nitrogen-phosphorus treatments in which the total potash contents of the leaves in terms of their alcoholic extract were slightly lower than in the check treatments. Among the fertilized plots, the total nitrogen content seems to be slightly less in the 6 per cent. ammonia treatment; but there appears to be no difference between the fertilizers containing 8 and 10 per cent. of this element. The total leaf phosphorus con-

TABLE III

TOTAL N, P_2O_5 AND K_2O IN LEAF IN PERCENTAGE OF DRIED ALCOHOLIC EXTRACT

TREATMENTS	N	P_2O_5	K_2O	TOTAL	K_2O/P_2O_5
	%	%	%	%	
(1) Check	7.747	0.861	8.29	16.898	9.63
(2) 6–8–10	9.135	2.128	14.13	25.393	6.64
(3) 8–10–0	10.579	3.272	6.77	20.621	2.07
(4) 8–10–10	9.642	2.283	13.51	25.435	5.92
(5) 8–10–15	10.196	2.304	14.11	26.610	6.12
(6) 10–8–0	10.929	2.945	8.05	21.924	2.73
(7) 10–8–10	10.501	2.538	15.43	28.469	6.08
(8) 10–8–15	10.924	2.435	14.61	27.969	6.00

tent in terms of the dried alcoholic extract is considerably higher in the minus-potash fertilizer treatments than in those containing potash. The result is entirely opposite to that obtained in the case of the phosphorus removed by the alcoholic extraction and calculated in terms of the dried extract. It is evident, therefore, that when potash was withheld from the fertilizer, the amount of phosphorus in the leaf increased. The reduced yield of this treatment is due to a deficiency of potash, or to a lack of equilibrium between phosphorus and potash. The last column of table III, shows that all complete fertilizer treatments, the yields of which are nearly equal, have practically the same potash-phosphorus ratio; whereas the incomplete treatments without potash show a very reduced potash-phosphorus ratio. This fact indicating that equally yielding treatments show a practically even ratio between phosphorus and potash, gives weight to the findings reported in the case of the alcoholic extract: irrespective of treatment, there was a constant potash-phosphorus ratio associated with the chlorophyll bodies in the synthetic processes of the leaves.

COMPOSITION OF THE ENTIRE LEAVES

No direct analysis was made of the whole leaves in the present study. But from the data obtained in this experiment as shown in tables I, II, and IV, the composition of the entire leaves was calculated and is shown in table V.

The nitrogen content of the entire leaves shows a relationship with the nitrogen content of the fertilizers applied. Its relationship with the yields resulting seems, however, to be dependent upon the contents of phosphorus and potash present for an adequate equilibrium. Check treatments without fertilizers showed the lowest nitrogen content in the leaves. Fertilizers with 6 and 8 per cent. of this nutrient increased the nitrogen content of the leaves but there seems to be very little difference between the 8 and 10 per cent. nitrogen treatments. The absence of potash from the fertilizer did not affect the content of nitrogen in the leaf but was substantially reflected in

TABLE IV

DRIED WEIGHTS OF ALCOHOLIC EXTRACT, LEAF SKELETON, AND ENTIRE LEAF; ALCOHOLIC EXTRACT AND LEAF SKELETON AS PERCENTAGE OF LEAF

TREATMENTS	LEAF SKELETON	ALCOHOLIC EXTRACT	ENTIRE LEAVES	ALCOHOLIC EXTRACT IN LEAVES	SKELETON IN LEAVES
	gm.	gm.	gm.	%	%
(1) Check	23.7205	8.3950	32.1155	26.14	73.86
(2) 6–8–10	13.8050	4.9395	18.7445	26.35	73.65
(3) 8–10–0	15.0235	4.9418	19.9653	24.75	75.25
(4) 8–10–10	14.9190	5.7945	20.7135	27.97	72.03
(5) 8–10–15	12.7190	4.6058	17.3248	26.58	73.42
(6) 10–8–0	24.5500	8.3414	32.8914	25.36	74.64
(7) 10–8–10	15.6690	5.6529	21.3219	26.51	73.49
(8) 10–8–15	21.5300	7.3783	28.9083	25.52	74.48

reduced yields when compared with the complete treatments. This points again to the fact that the efficiency of nitrogen as a plant nutrient depends upon its being equilibrated with the other essential elements.

The phosphorus content of the leaves was remarkably low in the check treatment. The addition of phosphorus to the fertilizer resulted in a direct increase of this nutrient in the leaves and also in greater yields. Consequently, there seems to be a direct relationship between phosphorus in the fertilizer, phosphorus in the leaves, and the yield. In the minus-potash treatments, the content of phosphorus in the leaves was substantially larger than in all the other cases under comparison. This is due to the fact that this nutrient was not utilized to its full extent owing to a lack of balance.

The potash content of the entire leaves is smaller in check treatments and in minus-potash treatments than in the complete fertilizer treatments. The application of potash to the crop was thus reflected in an increase of this element in the leaves and in higher yields. Consequently, there is a

TABLE V

PERCENTAGES OF NITROGEN, PHOSPHORUS, AND POTASH IN THE ENTIRE LEAVES OF IRISH COBBLER POTATOES

TREATMENTS	YIELD PER ACRE	NITROGEN N	PHOSPHORUS P_2O_5	POTASH K_2O	$N + P_2O_5 + K_2O$	K_2O/P_2O_5
	cwt.	%	%	%	%	
(1) Check	17.39	2.02	0.22	2.17	4.41	9.86
(2) 6–8–10	105.28	2.41	0.56	3.72	6.69	6.64
(3) 8–10–0	75.67	2.62	0.81	1.67	5.10	2.06
(4) 8–10–10	103.63	2.70	0.64	3.78	7.12	5.91
(5) 8–10–15	112.80	2.63	0.62	3.75	7.00	6.05
(6) 10–8–0	79.43	2.77	0.75	2.04	5.56	2.72
(7) 10–8–10	105.98	2.79	0.68	4.09	7.56	6.01
(8) 10–8–15	120.79	2.79	0.63	3.80	7.22	6.03

direct relationship between potash in the soil, potash in the leaves, and the resulting yields.

The sum total of nitrogen, phosphorus, and potash in the leaves is regarded by THOMAS (**5, 7**) as a factor expressing the intensity of nutrition of the crop. This factor is lowest in the check treatment which was also the lowest in yield. It was intermediate in the case of the minus-potash treatments which were also intermediate in yield. The complete treatments, on the other hand, were the highest in yield and at the same time the highest in the intensity-of-nutrition factor. Consequently, the intensity of nutrition measured in terms of the sum total of the percentages of nitrogen, phosphorus, and potash in the leaves of Irish potatoes bears a direct relationship to soil fertility on the one hand and to the resulting yields on the other. This confirms the findings of LAGATU and MAUME (**2, 3**), THOMAS (**5, 6, 7**), and THOMAS and MACK (**9, 10**).

From the statistical standpoint, no significant difference was found between the yields of the various complete fertilizer treatments. When the yields are correlated with the potash-phosphorus ratio in the leaves as shown in table V it is interesting to observe that the complete fertilizer treatments, which resulted in practically the same yields, also show very similar potash-phosphorus ratios. On the other hand, the check treatment shows a rather high potash-phosphorus ratio indicative of an acute phosphorus deficiency in the soil. Minus-potash treatments resulted in a very low potash-phosphorus ratio indicating a deficiency of potash in respect to the phosphorus applied. These results are identical to those obtained when the total K_2O and P_2O_5 in the leaves were expressed in terms of the dried alcoholic extract.

NITROGEN, PHOSPHORUS, AND POTASH IN THE ALCOHOLIC EXTRACT AND IN THE ENTIRE LEAVES AND THEIR RELATIONSHIP TO CROP YIELDS

As stated before, various investigators (**2, 5, 6**) use the term "intensity of nutrition" to designate the sum of the percentages of nitrogen, phosphorus, and potash in the dried leaves as a quantitative factor of the nutrition of crop plants. This same factor was applied in the present investigation to the composition of the alcoholic leaf extract as well as that of the entire leaves. In both cases, the intensity of nutrition showed a direct relationship to soil fertility on the one hand, and potato yields on the other. The term "N-P-K unit" is also used by the same investigators (**2, 5, 6**) to express the proportion which each one of these elements, reduced to milligram equivalents, bears to the sum total of their milligram equivalents, but multiplied by 100 to avoid fractions. This unit marks the equilibrium between the three elements at the moment when the leaf samples are taken. Table VI shows the yields obtained in the present work, the intensities of

nutrition, and the N-P-K units of both the alcoholic leaf extracts and the entire leaves of the various treatments.

Intensities of nutrition, calculated on the basis of the composition of the alcoholic leaf extract and of the entire leaves, differ in quantity but not in kind. The first one shows greater contrasts between the different treatments than the second. Both, however, indicate a direct relationship between fertilizers applied on the one hand and yields on the other; in both, the intensities of nutrition from similarly yielding treatments present little variation.

The N-P-K unit calculated from the composition of alcoholic leaf extract is higher in potash and lower in nitrogen than the corresponding values based on the composition of the entire leaves.

TABLE VI

INTENSITIES OF NUTRITION AND N-P-K UNITS BASED ON THE COMPOSITION OF ALCOHOLIC LEAF EXTRACT AND ENTIRE LEAVES OF IRISH COBBLER POTATOES

TREATMENTS	YIELD PER ACRE	ALCOHOLIC LEAF EXTRACT				ENTIRE LEAVES			
		INTENSITY OF NUTRITION	N	P	K	INTENSITY OF NUTRITION	N	P	K
	cwt.	%	%	%	%	%	%	%	%
(1) Check	17.39	3.45	31.15	6.67	62.18	4.41	44.41	9.33	46.26
(2) 6–8–10	105.28	11.22	23.41	6.55	70.04	6.69	62.55	8.57	28.88
(3) 8–10–0	75.67	5.24	43.88	5.86	50.26	5.10	72.80	13.22	13.88
(4) 8–10–10	103.63	12.34	22.81	6.92	70.27	7.12	64.20	9.00	26.80
(5) 8–10–15	112.80	12.83	21.87	6.42	71.71	7.00	61.74	9.40	28.86
(6) 10–8–0	79.43	7.38	40.96	4.40	54.64	5.56	72.46	11.62	15.92
(7) 10–8–10	105.98	12.83	22.26	6.47	71.27	7.56	63.25	9.09	27.66
(8) 10–8–15	120.79	11.75	27.09	7.55	65.36	7.22	64.94	8.62	26.44

It is interesting to observe that the N-P-K units of these two bodies are respectively practically alike in the complete fertilizer treatments, which, at the same time, show a high intensity of nutrition and result in more or less equal yields per acre. These facts may be interpreted as indicating that treatments giving rise to the same yields, may also result in like intensities of nutrition and N-P-K units. On the other hand, no fertilizer and incomplete fertilizer treatments which resulted in lower yields show a decreased intensity of nutrition in the alcoholic extract and in the entire leaves. Their N-P-K units in both cases are different from those existing in the case of complete treatments. In the latter, the N-P-K units in terms of both alcoholic leaf extract and entire leaves are higher in potash and lower in nitrogen than in the case of incomplete fertilizer treatments. In these the N-P-K units based on the alcoholic leaf extract are low in phosphorus and potash as compared to the corresponding magnitudes of complete fertilizer treatments. This confirms again the interrelationship between phosphorus and

potash in leaf metabolism. The N-P-K unit based on the entire leaves, however, shows that in the nitrogen-phosphorus treatments the phosphorus magnitude appears to be exceedingly high. As explained before, this is owing to the lack of potash; the excess of phosphorus beyond that needed to balance the available potash present remains unused in the leaf.

Summarizing, it may be said that treatments resulting in similarly high yields showed a high intensity of nutrition of more or less the same magnitude and practically equal N-P-K units calculated either on the basis of the composition of the alcoholic extract, or of the entire leaves. Treatments resulting in lesser yields gave rise to a lower intensity of nutrition and a derangement of the N-P-K unit. Thus when no potash was applied, the intensity of nutrition was low, and the alcoholic extract and entire leaf N-P-K units revealed higher nitrogen and lower potash proportions than when this nutrient was applied. Phosphorus, however, was low when potash was also low in the N-P-K units of the alcoholic leaf extract; it was exceedingly high in the N-P-K unit calculated on the basis of the entire leaves.

Calcium-magnesium-potash relationship

The percentages of calcium and magnesium in the alcoholic leaf extract, in the leaf skeleton, and in the entire leaves, are found in table VII. The contents of these elements in the entire leaves were calculated in the same manner described in the case of nitrogen, phosphorus, and potash.

Table VII indicates that both calcium and magnesium predominate in the leaf skeleton. In the alcoholic leaf extract the magnesium content is higher than that of calcium. It is interesting to observe that while the content of these two elements in the entire leaves of the check treament compares favorably with that of the complete fertilizer treatments, in the alcoholic leaf extract it is extremely low. Since the alcoholic extract of the

TABLE VII

Percentage of calcium and magnesium in the alcoholic leaf extract, leaf skeleton, and entire leaves

Treatments	Alcoholic leaf extract			Leaf skeleton			Entire leaves		
	CaO	MgO	Total	CaO	MgO	Total	CaO	MgO	Total
	%	%	%	%	%	%	%	%	%
(1) Check	0.111	0.191	0.302	1.59	1.65	3.24	1.20	1.27	2.47
(2) 6–8–10	0.133	0.559	0.692	1.96	0.88	2.84	1.48	0.80	2.28
(3) 8–10–0	0.175	0.708	0.883	1.92	1.94	3.86	1.48	1.64	3.12
(4) 8–10–10	0.118	0.716	0.834	1.72	1.01	2.73	1.27	0.93	2.20
(5) 8–10–15	0.118	0.532	0.650	1.15	1.33	2.48	0.87	1.12	1.99
(6) 10–8–0	0.148	1.084	1.232	2.04	1.33	3.37	1.56	1.26	2.82
(7) 10–8–10	0.133	0.841	0.974	1.86	0.95	2.81	1.40	0.92	2.32
(8) 10–8–15	0.111	0.646	0.757	1.38	1.04	2.42	1.06	0.93	1.99

check treatment was also low in nitrogen, phosphorus, and potash this fact may indicate that the utilization of these two elements is directly related to the three major elements essential for plant metabolism. This indication is further substantiated by the fact that the percentage of these nutrients in the alcoholic leaf extract, increases as the percentages of nitrogen, phosphorus, and potash in that body rise. There seems, however, to be a certain equilibrium between calcium and magnesium on the one hand and nitrogen, phosphorus, and potash on the other. When potash is withheld from the fertilizer, the contents of calcium and magnesium increase; this is evident not only in the alcoholic leaf extract, but also in the leaf skeleton and hence in the entire leaves.

Table VIII shows the equilibrium between calcium, magnesium, and potash in the alcoholic leaf extract and in the entire leaves. These figures are derived from the percentages of each one of these three elements in both bodies by reducing the values to milligram equivalents and finding the percentage of each in the sum of their respective milligram equivalents.

TABLE VIII

CALCIUM, MAGNESIUM, AND POTASSIUM EQUILIBRIUM* IN THE ALCOHOLIC LEAF EXTRACT AND IN THE ENTIRE LEAVES

TREATMENTS	ALCOHOLIC LEAF EXTRACT			ENTIRE LEAVES		
	Ca	Mg	K	Ca	Mg	K
	%	%	%	%	%	%
(1) Check	5.31	12.71	81.98	28.16	41.43	30.41
(2) 6–8–10	1.97	11.53	86.50	30.70	23.05	46.25
(3) 8–10–0	4.96	27.89	67.15	31.21	47.80	20.99
(4) 8–10–10	1.57	13.21	65.22	26.41	26.81	46.78
(5) 8–10–15	1.55	9.74	88.71	18.67	33.38	47.95
(6) 10–8–0	2.88	29.31	67.81	34.36	38.77	26.87
(7) 10–8–10	1.66	14.56	83.78	27.41	25 01	47.58
(8) 10–8–15	1.60	12.90	85.50	22.87	28.07	49.06

* Figures are derived from the percentages of each one of these three elements in both bodies by reducing the values to milligram equivalents and finding the percentage of each in the sum of their respective equivalents.

In the first place it is interesting to observe that in the Ca–Mg–K equilibrium in the alcoholic leaf extract, potash predominates and that the ratio of magnesium is higher than that of calcium. In the entire leaves the proportions of calcium and magnesium are higher than in the corresponding cases in the alcoholic extract.

In both bodies, alcoholic extracts and entire leaves, the ratios of calcium and magnesium increase as that of potash decreases and *vice versa*. This fact confirms the findings of THOMAS and MACK (**8**) who state that these basic elements calcium, magnesium, and potassium have certain functions in common and that when there is a marked deficiency of one the others may replace it.

Complete treatments which resulted in practically the same yields have very little variation in their calcium-magnesium-potassium equilibrium in the alcoholic extract and in the sum of calcium and magnesium as referred to potash in the entire leaves. In this body it is observed that treatments 4 and 7, with 10 per cent. potash, resulted in practically the same calcium-magnesium-potassium equilibrium. A deficiency of potassium in treatments 1, 3, and 6 and an excess of this nutrient in treatments 5 and 8 resulted in a derangement of that equilibrium. These facts indicate that the optimum absorption of potash in relation to nitrogen and phosphorus may result in an optimum equilibrium between the bases calcium and magnesium.

Discussion

The composition of the leaves of crop plants as a means of determining soil fertility deficiencies has gained widespread attention in recent years.

In the present paper the composition of the alcoholic leaf extract of Irish Cobbler potatoes is compared to that of the entire leaves of the same crop, with the object of determining their relative values as indices of soil fertility deficiencies, or the crop's nutrition.

The relationship between the nitrogen, phosphorus, and potash in the alcoholic leaf extract, the amounts of these nutrients applied in the fertilizers, and the corresponding resultant yields, is very enlightening. Additions of nitrogen, phosphorus, and potash in the fertilizer resulted in a direct increase of the content of these elements in the leaf extract and in corresponding increased yields. Consequently, the contents of the fertilizing elements in the alcoholic leaf extract are directly correlated with the amount of these elements applied and with the yields obtained. The total content of nitrogen, phosphorus, and potash, or the intensity of nutrition as applied to the alcoholic leaf extract, also showed a direct relationship to the fertilizers applied on the one hand and to the resulting yields on the other. The N-P-K units representing the equilibrium between the three elements were very similar in those treatments resulting in similar yields, but seemed to be deranged in those treatments resulting in smaller yields. This may be taken to indicate that treatments giving rise to similar yields show similar intensities of nutrition and N-P-K units calculated on the basis of the composition of the alcoholic leaf extracts.

Another point of great interest revealed by the analysis of the alcoholic leaf extract is the potash-phosphorus equilibrium. There seems to be a rather constant ratio between the contents of these two elements in that body. This constant ratio existed not only in complete fertilizer treatments but also in those without fertilizer and in incomplete (minus-potash) fertilizer treatments. When it is recalled that the alcoholic leaf extract comprises the total chlorophyll and other pigments of the leaves, this fact as-

sumes great significance. It indicates that potash and phosphorus are used in a definite proportion in the synthetic processes of the leaves.

In the case of the entire leaves, increments of nitrogen, phosphorus, and potash in the fertilizer resulted in direct increments of the contents of these nutrients in the entire leaves. The contrasts resulting from differential treatment, however, were not as marked as in the corresponding cases of the alcoholic leaf extracts. This was also evident in the intensity-of-nutrition factor calculated on the basis of the composition of the entire leaves.

The entire leaf N-P-K units differ from those of the alcoholic extracts in higher nitrogen and lower potash proportions. They resemble the latter, however, in that they are very similar in the cases of treatments resulting in equal yields and vary in treatments resulting in different yields.

The potash-phosphorus ratio in the entire leaves is very similar in treatments resulting in similar yields. In minus-potash fertilizer treatments the potash-phosphorus ratio decreased while in the case of check treatments representing an acute phosphorus deficiency the ratio between these elements increased.

In the present study either the composition of the alcoholic leaf extract or of the entire leaves reveals the nutrition of the crop and its relationship to field yields; the contrasts from the various differential treatments were more significant, however, in the case of the alcoholic leaf extract.

The calcium-magnesium-potassium relationships follow the same sequence in both the alcoholic extract and the entire leaves. The mineral composition of either of these bodies shows that when the potash content is high, that of calcium and magnesium (individually or combined) is low and *vice versa*. The fact that the CaO–MgO–K_2O unit, or better still the ratio $\frac{K_2O}{CaO + MgO}$, is nearly constant in both alcoholic leaf extract and entire leaves, in those treatments producing similar yields, indicates that under optimum conditions of soil fertility the plant absorbs these elements in a state of equilibrium.

Summary

The entire leaves and the alcoholic leaf extract of Irish Cobbler potatoes from various plots subjected to different fertilizer treatments were analyzed for nitrogen, phosphorus, potash, calcium, and magnesium. The fertilizer treatments consisted of 1500 pounds per acre of each of the following formulas: 6–8–10, 8–10–0, 8–10–15, 10–8–0, 10–8–10 and 10–8–15. Each of these fertilizer treatments resulted in increased yields as compared with check treatments; there was no significant difference, however, between the yields of the various complete fertilizer treatments.

Taking the check treatment as a basis for comparisons, the analysis of the alcoholic leaf extract showed direct relationships between the amounts of nitrogen, phosphorus, and potash in the fertilizer; the percentages of these

elements in the alcoholic leaf extract; and the resulting yields. The same relationship existed when the individual contents of these elements in the entire leaves were correlated with the fertilizer treatments on the one hand and the resulting yields on the other. The contrasts, however, between the various treatments were much more pronounced in the alcoholic extracts than in the entire leaves.

The sums of the percentages of nitrogen, phosphorus, and potash in the alcoholic leaf extracts and in the entire leaves were also used to study the intensity of nutrition. In both cases there was a very marked relationship between these percentages, the fertilizer treatments, and yields. Low yields, as in check plots, resulted in the lowest intensity of nutrition; intermediate yielding treatments, like the incomplete (minus-potash) treatments, resulted in intermediate intensities of nutrition; high yielding treatments, fertilized with complete mixtures, gave rise to the highest intensity of nutrition. The intensity-of-nutrition factors were very similar in the case of complete fertilizer treatments, the yields of which did not show any significant difference between one another. As in the case of the individual content of the various nutrients, the contrasts between the intensities of nutrition of the various treatments were more marked in the case of the alcoholic leaf extract than in the corresponding cases of the entire leaves.

The N-P-K units were used to study the equilibrium between the three major fertilizing elements in both the alcoholic leaf extract and the entire leaves. Complete fertilizer treatments resulting in similar yields showed similar N-P-K units in both bodies. The N-P-K units on the entire leaf basis, however, were high in nitrogen whereas on the basis of the alcoholic leaf extracts they were high in potash.

Incomplete (minus-potash) fertilizer treatments resulted in a derangement of the N-P-K units in both bodies studied. This derangement was characterized by a decrease in the potash ratio and an increase in the nitrogen ratio.

Of particular significance is the fact that the alcoholic leaf extract showed a rather constant potash-phosphorus ratio irrespective of the widely different treatments under comparison. Thus, when the potash content of this body was low, its phosphorus content was also low in spite of the fact that a rather high phosphorus application was made. In a similar fashion, when the content of potash in the alcoholic extract was high, that of phosphorus was also high. In both cases the ratios between these two elements appeared to be rather constant. This may be construed to indicate that there is a definite equilibrium between potash and phosphorus in the synthetic processes of the leaves; as a result of this, the excess of any one of these elements beyond the amount which is in equilibrium with the other remains unused in the leaf. This condition is reflected by the analysis of the entire leaves. In the present study, the no-potash treatments showed a very high phos-

phorus content in the leaves since, in the absence of potash, the absorption of phosphorus increased even though it was utilized only in proportion to the quantity of potash available to equilibrate it.

Both the alcoholic leaf extract and the entire leaves show the same sequence in the calcium-magnesium-potassium relationships. When potash was high, calcium and magnesium, individually and collectively, were low, and *vice versa*.

The analysis of the entire leaves of potatoes, as well as that of the alcoholic leaf extract, may be used as indices of soil fertility deficiencies, or as indicators of the nutrition of the crop. When comparing soils differing widely in fertility, either one or the other may be used but when the fertility level between soils under comparison is not very pronounced, better results will be obtained by using the alcoholic leaf extracts.

MANZANA DE GOMEZ 516
HAVANA, CUBA

LITERATURE CITED

1. BEAUCHAMP, C. E. The mineral composition of the alcoholic extract of potato leaves and its relationship to crop yields. Plant Physiol. **15**: 485–502. 1940.
2. LAGATU, H., and MAUME, L. The variations of the sum $N + P_2O_5 + K_2O$ per 100 parts of dry material of the leaf of a cultivated plant. Compt. Rend. Acad. Agr. France **21**: 85–92. 1935.
3. ————, and ————. The sensitivity of foliar diagnosis for revealing fertilizer absorption by the vine (grape). Compt. Rend. Acad. Agr. France **24**: 615–624. 1938.
4. LAZO, F., and BONAZZI, A. Studies in sugar cane physiology. IV. Phosphorus in cane. Proc. Seventh Ann. Conf. Cuban Assoc. Sugar Cane Techn., pp. 75–82. December, 1933.
5. THOMAS, W. Foliar diagnosis: Principles and practice. Plant Physiol. **12**: 571–599. 1937.
6. ————. Foliar diagnosis: Its relation to the optimum nutrition of the potato. Plant Physiol. **13**: 677–694. 1938.
7. ————. Foliar diagnosis: Application of the concepts of quantity and quality in determining response to fertilizers. Proc. Amer. Soc. Hort. Sci. **35**: 269–272. 1938.
8. ————, and MACK, WARREN B. Foliar diagnosis: Physiological balance between the bases lime, magnesia, and potash. Plant Physiol. **14**: 699–715. 1939.
9. ————, and ————. The foliar diagnosis of *Zea mays* subjected to differential fertilizer treatment. Jour. Agr. Res. **58**: 477–491. 1939.
10. ————, and ————. Control of crop nutrition by the method of foliar diagnosis. Pennsylvania Agr. Exp. Sta. Bull. 378. 1939.

EFFECT OF TEMPERATURE ON THE RESPIRATION RATE AND THE RESPIRATORY QUOTIENT OF SOME VEGETABLES[1]

HANS PLATENIUS

(WITH SEVEN FIGURES)

Introduction

Numerous experiments have been conducted to determine the respiration rates of various vegetables after harvest. Nevertheless, it is well-nigh impossible to gain a clear knowledge of the comparative rates of respiration exhibited by different kinds of vegetables from the scattered data in the literature, because of the wide variations in temperature and other conditions under which such experiments were carried out. Only the studies of BENOY (**4**) and of APPLEMAN and SMITH (**1**) represent consistent efforts to secure comparable data on respiration under uniform conditions.

Aside from transpiration, respiration is undoubtedly the most important factor contributing to the deterioration of vegetables after harvest. Additional information on respiration rates at different temperature levels is needed for the solution of practical problems concerned with storage and transportation of fresh vegetables. The experiments here reported were carried out with this point in view. Carbon dioxide production and oxygen consumption were determined simultaneously in the hope that the calculated respiratory quotients would shed some light on the metabolism involved. In particular, it was expected that an explanation might be found for the phenomenon of low temperature injury which has frequently been observed in certain fruits and vegetables and which has been attributed to abnormalities in the course of respiration.

Methods

The procedure followed was to measure simultaneously the rate of oxygen uptake and carbon dioxide evolution, and was essentially the same as that developed by MAGNESS and DIEHL (**12**). Suggestions for the improvement of this method offered by HALLER and ROSE (**9**) were followed and the writer further changed the apparatus by applying the principle of the Mariotte bottle in order to maintain a uniform atmospheric pressure in the system.

Depending on the temperature of the room and the respiratory activity of the vegetable under examination, a representative sample, varying from 200 to 3000 gm., was placed in the respiration chamber (E) (fig. 1). After

[1] Paper no. 236. Department of Vegetable Crops, Cornell University, Ithaca, New York.

applying the cover, the air in the chamber was saturated with water vapor by means of a hand atomizer. Through the funnel (D) 50 ml. of 1 N sodium hydroxide were added to the funnel (F). The chamber was then connected to the oxygen cylinder (B) and this in turn was attached to the Mariotte bottle (A).

Carbon dioxide produced in the course of respiration was absorbed by the alkali in the bottom of the chamber. As oxygen was consumed by the vegetables, the gas pressure in the chamber diminished and this caused a flow of oxygen from the cylinder (B) into the chamber. Atmospheric pressure in the entire system was quickly reestablished by the flow of water from the Mariotte bottle into the oxygen cylinder. This arrangement proved to be so sensitive that a pressure deficit of only one or two mm. of water as read on the manometer (H) was sufficient to cause water to enter the cylinder. It should be pointed out that the actual height of the water level in the

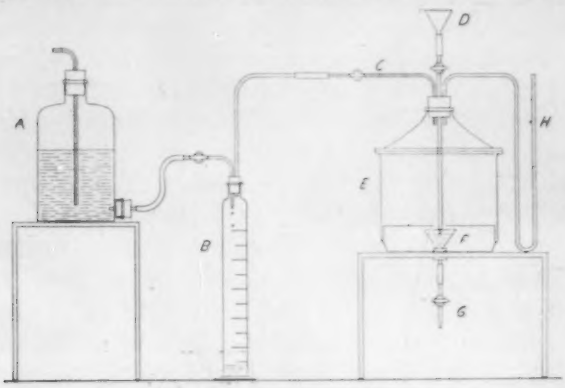


FIG. 1. Apparatus used to measure simultaneously oxygen consumption and carbon dioxide production during respiration.

Mariotte bottle has no effect on the pressure in the system and atmospheric pressure is maintained as long as the lower tip of the capillary tube in A is kept at the same level as the tip of the delivery tube entering the cylinder (B).

At the end of each run the quantity of oxygen displaced by water in the cylinder (B) was measured. At the same time, the alkali in the funnel (F) was drawn off, an excess of barium chloride was added, and the amount of carbonate present determined by double titration against 1 N hydrochloric acid using phenolphthalein and methyl orange as indicators.

A serious error in the oxygen readings arises from the fact that changes in atmospheric pressure and temperature produce volume changes in the system which at low temperatures occasionally exceed those resulting from the consumption of oxygen by the vegetables. Since there is no simple method whereby a uniform atmospheric pressure can be maintained, correc-

tions had to be applied. This was done by setting up a second chamber of the same size and thickness of glass as the chamber (E). Pressure changes occurring during each run were recorded on the gauge of this control chamber; thus it was possible to calculate accurately necessary corrections for the volume of gases occupying the respiration chamber and oxygen cylinder. The volume of oxygen consumed was calculated to standard conditions on the basis of the atmospheric pressure and temperature prevailing at the beginning of each run.

Another source of error was eliminated by allowing equilibrium to establish itself between the carbon dioxide in the chamber and the absorbing alkali before the initial oxygen reading was taken. Actually, a true equilibrium is never reached because the absorbing power of the hydroxide diminishes as its concentration decreases. Consequently, the partial pressure of carbon dioxide in the chamber increases throughout the run. Preliminary experiments showed, however, that during the first four hours the concentration rose to one per cent. or less, depending on the actual rate of respiration. Thereafter, the rate of increase in the partial pressure of carbon dioxide became so small that it could be neglected. In the course of actual experimentation, the funnel (F) was drained four hours after the apparatus was set up and fresh sodium hydroxide was added after which the initial reading was taken.

The corrected value for the volume of oxygen consumed in respiration was calculated according to the formula:

$$V_o = V_1 + KV_1 + \frac{P}{P_1} \times V_e \times \frac{P_1 - PH_2O}{760} \times \frac{273}{T}$$

where V_o = corrected volume of oxygen reduced to standard conditions.

V_1 = volume of oxygen displaced by water.

V_e = free volume of the system at the end of the experiment.

K = constant to correct for differences in the solubility of oxygen and nitrogen in water when in equilibrium with air and pure oxygen at the temperature T.

P = pressure change as measured in the check chamber.

P_1 = initial barometric pressure.

PH_2O = vapor pressure of water at the temperature T.

T = temperature in degrees of absolute temperature.

Respiration studies were carried out in three rooms in which temperatures of 0.5, 10.0, and 24.0° C. were maintained. In order to keep temperature fluctuations within 1° C., it was found necessary to enclose the apparatus in a large box well insulated with fiber board. Light was excluded from the vegetables except for brief periods when the apparatus was assembled or when readings were taken.

Immediately after harvesting the vegetables were cleaned, weighed, and aliquot lots distributed in the three rooms. Usually the first determinations of the respiration rate were made 6 hours after harvest. There were two

exceptions, however. For greenhouse cucumbers, which were obtained from a grower in Indiana, the first determination was delayed three days. Potatoes used in these experiments had been harvested three weeks earlier and in the meantime they had been held at an approximate average temperature of 13° C.

Depending on the kind of vegetable involved and the temperature of the storage room, each respiration run lasted from five hours to three days. During this period, the vegetables were necessarily exposed to an atmosphere saturated with water vapor. Between experiments, they were kept in the same room and at the same temperature, but at a relative humidity considerably below that of the respiration chambers. In some vegetables, this resulted in severe wilting, a fact which must be taken into account when interpreting the results of these respiration studies. Depending on the expected storage life at each of the three temperatures, determinations of the respiration rate were repeated at intervals varying from one day to one month until the samples showed signs of internal breakdown or infection with disease organisms.

All respiration data were expressed on the basis of the original fresh weight. It was feared that calculations on the basis of fresh weight at successive periods would yield misleading results, since changes in the fresh weight are caused primarily by the loss of water in transpiration, whereas the actual dry weight of the samples decreases but little.

The kinds of vegetables and the particular variety used in these respiration studies were as follows:

Asparagus (*Asparagus officinalis*); var. Mary Washington.
Peas (*Pisum sativum*); var. Laxton Progress.
Snap beans (*Phaseolus vulgaris*); var. Tendergreen.
Spinach (*Spinacia oleracea*); var. Long Standing Bloomsdale.
Lettuce (*Lactuca sativa*); var. New York (Imperial 44).
Carrots (*Daucus carota*); var. Red Core Chantenay.
Peppers (*Capsicum frutescens*); var. Windsor A.
Tomatoes (*Lycopersicum esculentum*); var. Marglobe.
Cucumbers (*Cucumis sativus*); var. Davis Perfect.
Potatoes (*Solanum tuberosum*); var. Rural.

It will be noticed that this list includes vegetables representing different plant organs such as shoots, roots, tubers, fruits, and leaves. Also, these vegetables have a wide range of respiratory activity, some having a respiration rate more than 50 times as high as others.

Results and discussion

Factors affecting the respiration rate

Following the conventional method of expressing the degree of respiratory activity, the results of these experiments are reported in terms of car-

bon dioxide production. Calculations based on oxygen consumption would have led to the same general conclusions with respect to the relative respiration rates of the different vegetables. Considering first the rates at 24° C., shown in figures 2 and 3, it will be noticed that in general the rates declined rapidly during the first few days of storage. Thereafter, the trend became irregular, some vegetables showing a further decrease, others an upward trend in respiratory activity. Striking differences are apparent in the relative rates of the ten vegetables studied. To mention two extremes, the initial rate of asparagus was found to be 59 times as high as that of potatoes. The trends in respiratory activity at 10° C. are shown in figures 4 and 5 and those at 0.5° C. in figures 6 and 7. While the decline in respiration rates followed the same pattern as it did at 24° C., the general level of activity became decidedly lower as the storage temperature decreased.

Unquestionably, the actual respiration rate observed in each instance is the integrated result of several factors, but an explanation of the behavior of any one vegetable must rest on pure speculation. Nothing more is possible than to point out some of the factors which appear to play a prominent rôle in the respiration process.

While most of the available evidence supports the theory that, of the different forms of sugar found in plant material, only glucose serves as the ultimate substrate in respiration, data have been presented by BARKER (**2**) which suggest strongly that, in potatoes at least, the gamma-fructose part of the sucrose molecule is oxidized before glucose enters the respiration process. Regardless of the types of sugar which may serve as substrates, the observed differences in the initial respiration rate of different vegetables cannot be explained on the basis of the law of mass action. Examination of table I furnishes convincing proof that no correlation exists between respiratory activity of different vegetables and their content of either glucose or total sugars. This lack of correlation is by no means to be taken as evidence that the law of mass action plays no rôle in determining respiration rates; its effect may merely be masked by the influence of other factors of greater importance. In considering the respiration curves of individual vegetables, many of the internal factors remain nearly constant in their effect on respiration and it becomes then possible to explain the gradual decline in respiration rates by means of the law of mass action. Still, the view may be taken that this decline in rate is the effect of gradual aging and slow disintegration of the cells.

Little is known about the kinds and quantities of enzymes taking part in the respiration process directly or indirectly. It seems likely that this factor is the most important one of all. In fact, differences in the respiratory activity of the various plant organs may in the last analysis be attributed to deviations in enzyme activity.

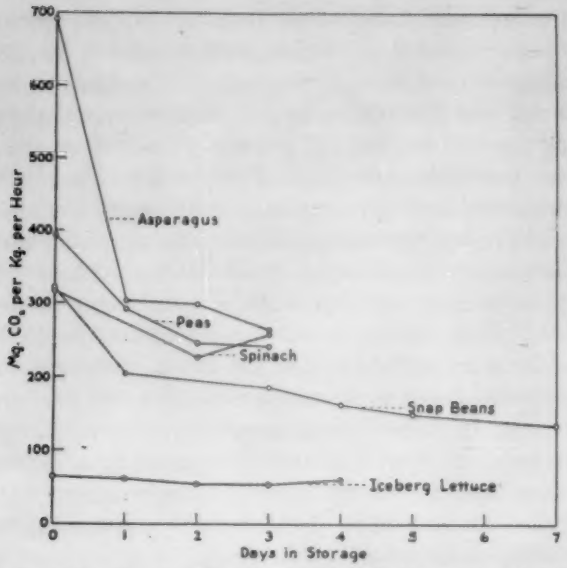


FIG. 2. Rate of carbon dioxide evolution during storage at 24° C.

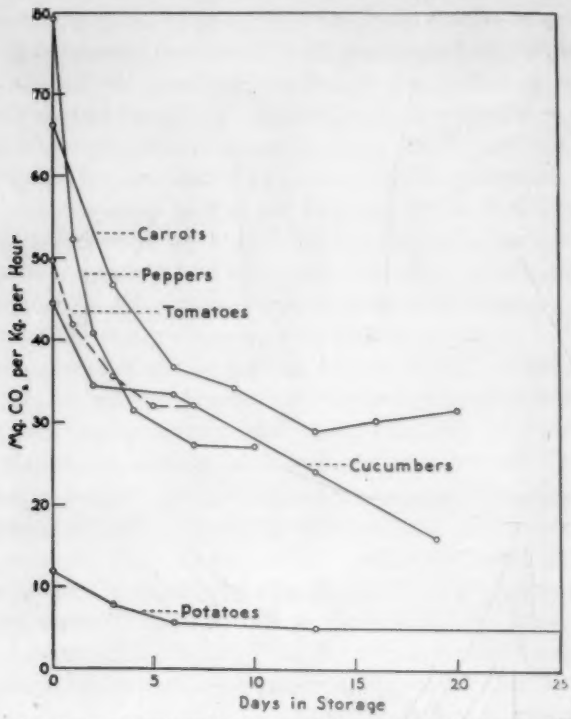


FIG. 3. Rate of carbon dioxide evolution during storage at 24° C.

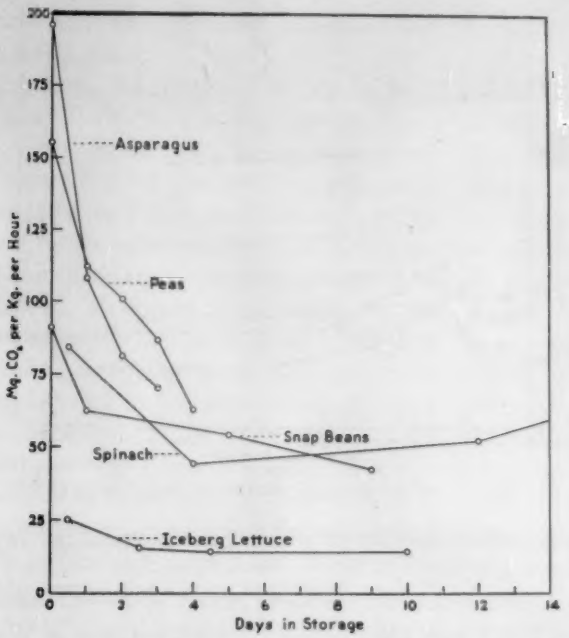


FIG. 4. Rate of carbon dioxide evolution during storage at 10° C.

A factor of minor importance is the permeability of the epidermis to carbon dioxide and oxygen. Because of the dissimilarity in the epidermal

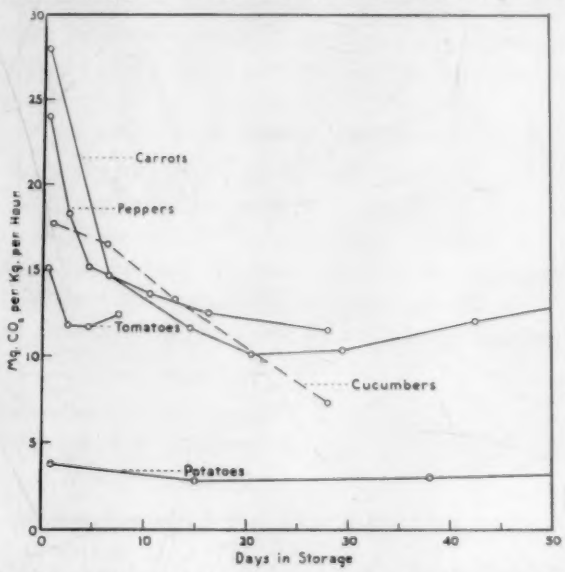


FIG. 5. Rate of carbon dioxide evolution during storage at 10° C.

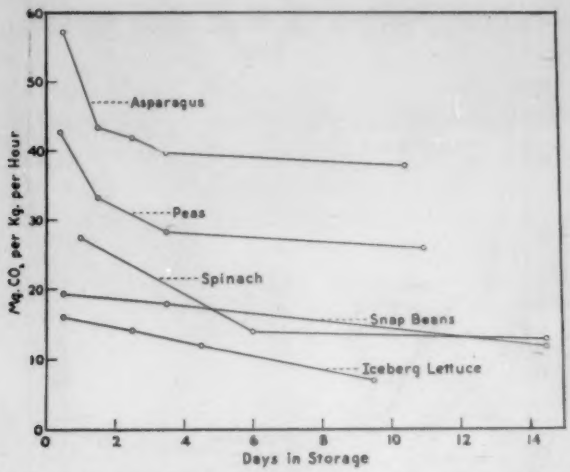


FIG. 6. Rate of carbon dioxide evolution during storage at 0.5° C.

structure of vegetables the rate at which gaseous exchange can take place should vary correspondingly.

It must be admitted that little is to be gained by merely enumerating the various factors which may influence the respiration rate of plant material. Not until many additional data become available to show quantitatively the relationship between each one of these factors and respiration rate will it be

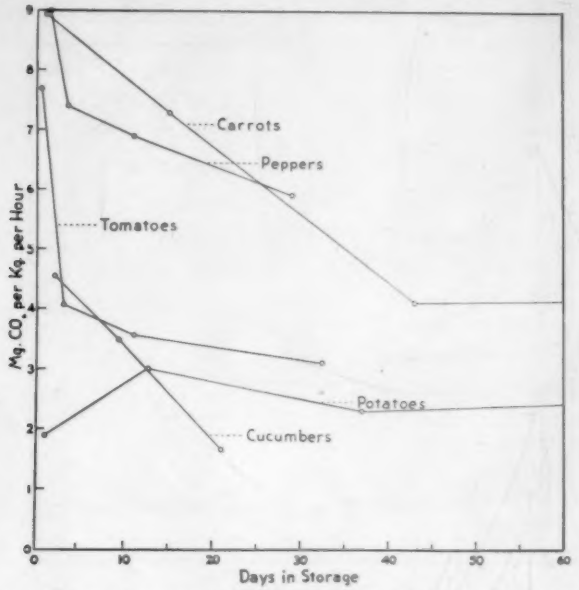


FIG. 7. Rate of carbon dioxide evolution during storage at 0.5° C.

possible to state with any degree of assurance why the wide variability in respiratory activity exists.

As stated before, the respiration rate falls off as the storage period progresses. This decline is most rapid during the first few days and usually diminishes as time goes on; also, the decrease in respiratory activity is more rapid at the higher than at the lower temperatures. Regardless of whether this decline is attributed to mass action or to other physiological processes associated with the aging of the tissue, it is interesting to find that the general trend of the respiration curves can easily be upset when a new factor becomes operative. Instances of this type occurred during the storage of lettuce, spinach, carrots, and potatoes at 24° C. and to some extent at 10° C. While the respiration rate of these vegetables declined during the first few days a subsequent rise in the rate became apparent toward the latter part of the storage period. At first it was believed that this secondary rise in spinach and lettuce might be attributable to the growth of microorganisms. Close examination, however, showed them to be free of decay spots. Instead it was found that the increase in respiration activity was associated with new growth. Both lettuce and spinach had begun to develop new growing points which probably would have developed into seedstalks had it been possible to prevent ultimate breakdown from other causes. Similarly, carrots and potatoes had begun to produce sprouts at the time the decline in respiration rate had ceased. It was also noticed that changes in the trend of respiratory activity did not occur until the sprouts had reached a length of two centimeters or more. These observations make it evident that the secondary rise in respiration rate is the result of the high rate of metabolic activity of the newly developed tissue itself and not the effect of the mere initiation of new growth.

Substrate used in respiration

Although no attempt was made to trace metabolic changes associated with respiration in these experiments, it is possible to make certain deductions from the available data. The respiration experiments of Asparagus were accompanied by chemical analyses of comparable samples taken at harvest time and again after three days of storage at 24° C. The following table shows the results of the analyses for total sugar content:

	Grams total sugar per 100 grams original fresh weight
At the beginning of the experiment	1.87
After three days of storage at 24° C.	1.06
Loss in total sugars	0.81

Sugar losses were compared with the amount of glucose corresponding to the carbon dioxide produced in Asparagus held under identical conditions for the same interval. From the data given in figure 2, it was calcu-

lated that during this period 2.57 gm. of carbon dioxide were produced per 100 gm. of fresh material and this quantity is equivalent to 1.75 gm. of glucose. Obviously, the quantity of total sugars lost can account for only one-half of the carbon dioxide given off. The conclusion seems inevitable that a large share of this substrate was furnished by substances other than sugars. The discrepancy between sugar losses and carbon dioxide evolution is further aggravated by the fact that, according to BISSON and JONES (**5**), there is a substantial gain of crude fibre in storage and this gain must be ascribed to a polymerization of sugars, a factor which tends to increase still further the quantity of the sugar equivalent of carbon dioxide unaccounted for. Starch being absent in Asparagus and fats being available in minute quantities only, one must conclude that protein was utilized in respiration to a considerable extent.

Many textbooks on plant physiology contain statements to the effect that proteins may undergo decomposition and become available for respiration, but it is generally assumed that this process occurs only if plants are in a starved condition; that is, when all or most of the available carbohydrate reserve has been consumed. The present data suggest that one-half of the substrate was furnished by the hydrolysis of proteins and deaminization of amino-acids at a time when more than one-half of the original sugar content was still present. Further evidence in support of the theory of protein utilization is given by the low values for the respiratory quotient, shown in table III, which at 24° C. dropped to less than 0.9 after the first day and rose again to 0.95 on the last day of storage. Depending on their particular composition, proteins give a respiratory quotient of 0.80 to 0.82. Proteins and sugars being utilized in respiration at equal rates would give a quotient of 0.9 or slightly higher, a value which agrees reasonably well with the quotient actually observed.

The metabolism of Asparagus in storage is not a unique case, however. On the basis of detailed chemical analysis, YEMM (**17**) proved conclusively that some of the protein in detached barley leaves held in the dark was decomposed and utilized in the respiration process during the first 12 hours of storage, long before the available carbohydrate reserve was depleted. VICKERY, PUCHER, WAKEMAN, and LEAVENWORTH (**16**) obtained indirect evidence of the utilization of leaf proteins of rhubarb during respiration, and CHIBNALL (**6**), discussing protein metabolism in connection with respiration, comes to the conclusion that protein destruction and the utilization of the products of hydrolysis in respiration occurs more frequently and at an earlier stage than had been heretofore supposed.

As illustrated in table I, neither the concentration of sugars nor that of total carbohydrates in the various vegetables is related to their initial respiration rates. A comparison of the respiration curves for peas and snap beans further shows that sugar concentration alone does not materially alter

TABLE I

GLUCOSE, TOTAL SUGARS, AND TOTAL CARBOHYDRATE CONTENT OF TEN VEGETABLES ON A FRESH WEIGHT BASIS AND THEIR INITIAL RESPIRATION RATE AT 24° C.*

VEGETABLE	GLUCOSE	TOTAL SUGARS	TOTAL CARBOHYDRATES	RESPIRATION RATE PER HOUR PER KG.
	%	%	%	$mg. CO_2$
Asparagus	1.80	1.9	1.9	692.0
Peas	0.90	6.3	8.3	394.0
Snap beans	2.50	2.7	3.8	321.0
Spinach	1.20	3.0	4.1	318.0
Peppers	?	2.1	6.3	78.8
Carrots	3.02	6.8	6.8	66.2
Iceberg lettuce	?	2.2	2.2	64.2
Tomatoes	3.60	3.6	3.7	49.6
Cucumbers	1.90	2.6	2.6	44.5
Potatoes	0.30	0.9	15.6	11.8

* Data compiled from various sources in the literature and from unpublished analyses obtained by the writer.

the rate at which the respiratory activity decreases in any one vegetable (fig. 2). As is generally known, the sugar content of peas at 24° C. is rapidly depleted, whereas PARKER and STUART (**14**) demonstrated that the percentage of sugars in snap beans remains nearly constant. In spite of the wide differences in the sugar concentration of these two vegetables after several days of storage, the rate at which the respiration rate of each diminished was nearly the same. Potatoes shifted from a temperature of 12° C. to 0.5° (fig. 7) behaved differently; at first the respiration rate increased, then dropped again and assumed a nearly constant level after 37 days. Based on the results of HOPKINS (**10**), one could attribute the initial rise in respiration rate to the shift of starch to sugar which takes place at that temperature. APPLEMAN and SMITH (**1**), however, found that potato tubers shifted from a low temperature to 30° C. had a high initial respiration rate which declined rapidly in spite of the fact that at this temperature the concentration of total sugars actually increased for 10 days. All available evidence makes it extremely doubtful, therefore, that a shift in the sugar-starch equilibrium of plant material has a direct effect on the respiration rate.

TEMPERATURE COEFFICIENTS

The relationship between temperature and respiration rate is usually expressed in terms of Q_{10} values which indicate the ratio between reaction rates at intervals of 10° C. Since in these experiments, temperature intervals were 9.5° and 14.0° C., respectively, it became necessary to recalculate the data on the basis of a 10° temperature range. This was done by applying the formula

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}}$$

where T_1 and T_2 are the lower and upper temperature levels and R_1 and R_2 the corresponding respiration rates based on carbon dioxide production. Using this method, it was possible to compare the acceleration of the respiration rate with temperature for different temperature ranges.

There are certain limitations in the use of Q_{10} values for respiration rates. First of all, such values, in order to have any meaning, can be applied to the initial rates only, since in comparisons at any later stage one would be dealing with vegetables of different physiological age and different chemical composition. Furthermore, one must remember that temperature coefficients expressed as Q_{10} values represent averages only and there is good reason to believe that within the observed range the rate of acceleration is by no means uniform.

In table II, the Q_{10} values for two temperature ranges are arranged in descending order of respiratory activity of the vegetables as determined for the initial rate at 24° C. It is obvious that there is a complete lack of correlation between the initial respiration rate at a given temperature and the effect that a change in temperature produces on respiratory activity. The

TABLE II

Q_{10} VALUES FOR RESPIRATION OF TEN VEGETABLES

Vegetable	Temperature range	
	0.5°–10.0° C.	10.0°–24.0° C.
Asparagus	3.7	2.5
Peas	3.9	2.0
Snap beans	5.1	2.5
Spinach	3.2	2.6
Peppers	2.8	2.3
Carrots	3.3	1.9
Iceberg lettuce	1.6	2.0
Tomatoes	2.0	2.3
Cucumbers	4.2	1.9
Potatoes	2.1	2.2

magnitude of the Q_{10} values within the range of 0.5° to 24° C. varies from 1.6 to 5.1, indicating that VAN'T HOFF'S rule applies to respiration rates only very roughly. Still, this range of Q_{10} values is much narrower than that found for chemical reactions involving carbohydrate metabolism in vegetables where the writer found values as high as 27.5 (**15**).

The calculated Q_{10} values vary not only with different vegetables, but also with the temperature range at which they were determined. In seven out of ten vegetables, the values were lower at the upper temperature range. This relationship is in accord with the general observation that the rate of acceleration of biological reactions diminishes as higher temperatures are approached. On the other hand, the findings stand in contrast to the results

of GERHART (7) and GORE (8) who, working with various fruits, failed to notice any decided falling off in the acceleration of respiration until a temperature of 32° C. was attained.

In interpreting the calculated Q_{10} values, it is well to distinguish between the direct and indirect temperature effects. The rate of increase in respiration with rising temperature would probably follow a comparatively smooth curve were it not for the fact that any shift from one temperature to another involves pronounced changes in plant metabolism. Such changes do not necessarily follow VAN'T HOFF'S rule; in fact, a complete reversal in the reaction equilibrium may take place as is illustrated by the sugar-starch equilibrium of potato tubers at different temperatures. Little is known of the extent to which shifts in the metabolic course with changing temperature influence the respiration rate. One may reasonably assume, however, that the existing variations in the Q_{10} values found are in part caused indirectly by the effect that temperature changes have on plant metabolism.

RESPIRATORY QUOTIENT AND SUBSTRATES USED IN RESPIRATION

Certain deductions concerning the course of respiration can be made if the available data show not only the rate of carbon dioxide production, but also the quantity of oxygen consumed. In fact, much valuable information about the mechanism of plant respiration has been obtained from experiments in which the respiratory quotient was the principal criterion of the effect that certain factors, such as anesthetics, had on the respiratory quotient under controlled conditions.

In the present study, calculations of the respiratory quotient were included primarily for the purpose of ascertaining to what extent substrates, other than carbohydrates, were involved in the respiration process. It must be admitted, however, that the respiratory quotient alone cannot give any definite information concerning either the course of respiration or the substrate utilized. In no way can the magnitude of the respiratory quotient be considered an expression of the degree to which the oxidation process has been completed. Depending on whether organic acids or fats and proteins serve as substrates, a quotient above or below one may be obtained by complete oxidation to carbon dioxide and water. Likewise, under partial anaerobic conditions some of the sugar may be converted to alcohol and carbon dioxide or it may be oxidized to organic acids; the corresponding respiratory quotients would be above one in the former, and below one in the latter case. The interpretation of the respiratory quotient is further complicated by the fact that several substances may be involved in the respiration process simultaneously. If, for instance, proteins, sugars and organic acids serve in equal molar proportions as substrates for the complete oxidation to carbon dioxide and water, the observed quotient would be close to

unity, a value similar to that which would be obtained if only sugars were involved in the respiration process. Consequently, it is possible to draw definite conclusions from the observed respiratory quotients only if the studies are supplemented by detailed chemical analyses serving as a balance sheet to show exactly the net loss or gain in the substrates and end-products of respiration.

The data in tables III, IV, and V are presented, therefore, only for the purpose of suggesting instances where an unusual behavior in the respiration process is indicated. Subject to a few exceptions, certain trends are apparent. For most vegetables, a quotient fairly close to unity was observed. In general, the respiratory quotient was highest immediately after harvest. As the storage period progressed, the quotient drifted to slightly lower levels. A definite relationship between the storage temperature and the respiratory quotient was also indicated; higher values were usually obtained in the upper temperature range.

Considering the behavior of each vegetable separately, the possibility suggests itself that the respiratory quotient in some way is connected with the intensity of respiration. It is not unlikely that rapid utilization of organic acids occurs whenever the rate of respiration is high, which would result in a quotient above unity. It may also be that protein decomposition plays an increasingly greater rôle toward the end of the storage period, tending to depress the quotient obtained. Detailed chemical analyses of plant tissue in storage are required before any such causal relationship can be established as fact.

Some speculation may be permitted to explain the behavior of those few crops that show marked deviations in their respiratory quotients from unity. The behavior of Asparagus has been discussed earlier. Two other vegetables showing even more pronounced abnormalities are spinach and potatoes, both having unusually low quotients, especially at 0.5° C. The unusual behavior of spinach may be explained by assuming that leaf protein was decomposed and utilized in respiration to a large extent. There is also a remote possibility that during storage, part of the oxygen was used in the f.rmation of organic acids. According to compilations by KOHMAN (**11**) the oxalic acid content of fresh spinach may vary from 0.29 to 0.69 per cent. It was calculated that the excess quantity of oxygen consumed during a 10-day storage period at 0.5° C. would yield only 0.1 gm. of additional oxalic acid for each 100 gm. of fresh weight.

No definite explanation can be offered for the unusually low quotients exhibited by potatoes in storage. The values obtained agree closely with those of BENNETT and BARTHOLOMEW (**3**) who calculated quotients as low as 0.49 for tubers stored at 7.5° C. In both sets of data, the lowest values were found at the beginning of the storage period; later, a gradual increase

TABLE III

RESPIRATORY QUOTIENT OF TEN VEGETABLES IN STORAGE AT 24° C.

VEGETABLE	DAYS IN STORAGE												
	0	1	2	3	4	5	6	7	8	10	12	19	30
Asparagus	1.04	0.89	0.88	0.95	.....	.....	.....	.....	.....	.....	.....	.....	.....
Peas	1.32	1.05	1.05	1.06	.....	.....	.....	.....	.....	.....	.....	.....	.....
Snap beans	1.14	1.01	.....	1.00	0.99	1.01	.....	1.01	.....	.....	.....	.....	.....
Spinach	0.94	.....	0.85	0.83	.....	.....	.....	.....	.....	.....	.....	.....	.....
Peppers	1.12	1.02	0.97	.....	0.90	.....	.....	0.83	.....	0.88	.....	.....	.....
Carrots	1.10	.....	.....	1.05	.....	.....	1.07	.....	1.07	.....	1.10	1.18	.....
Iceberg lettuce	1.12	1.05	1.07	0.99	1.02	.....	.....	.....	.....	.....	.....	.....	.....
Tomatoes	1.11	1.13	1.11	1.09	.....	1.12	.....	1.13	.....	.....	.....	.....	.....
Cucumbers	1.01	.....	1.07	.....	.....	.....	1.15	.....	.....	.....	1.08	0.91	.....
Potatoes	1.02	.....	.....	0.83	.....	.....	0.79	.....	.....	.....	0.75	.....	0.91

TABLE IV

RESPIRATORY QUOTIENT OF TEN VEGETABLES IN STORAGE AT 10° C.

VEGETABLE	DAYS IN STORAGE										
	0	1	2	3	4	5–7	10–12	13–18	30–40	41–60	61–80
Asparagus	1.03	0.93	0.83	0.86	.....	.....	.....	.....	.....	.....	.....
Peas	1.13	1.11	1.07	1.05	1.00	.....	.....	.....	.....	.....	.....
Snap beans	1.08	0.98	.....	.....	.....	1.01	1.00	.....	.....	.....	.....
Spinach	0.90	.....	.....	.....	0.87	.....	0.84	0.86	.....	.....	.....
Peppers	1.27	.....	1.01	.....	1.02	.....	1.01	0.91	0.88	.....	.....
Carrots	1.08	.....	.....	.....	.....	0.89	.....	0.99	0.94	1.01	1.01
Iceberg lettuce	1.09	.....	0.93	.....	0.94	.....	1.00	.....	.....	.....	.....
Tomatoes	1.39	.....	1.08	.....	1.04	1.06	.....	.....	.....	.....	.....
Cucumbers	1.01	.....	.....	.....	.....	1.03	.....	1.06	1.10	.....	.....
Potatoes	.....	0.86	.....	.....	.....	.....	.....	0.83	0.86	.....	0.99

TABLE V

RESPIRATORY QUOTIENT OF TEN VEGETABLES IN STORAGE AT 0.5° C.

VEGETABLE	DAYS IN STORAGE											
	1	2	3	4–6	6–8	8–10	11–15	16–20	21–30	31–40	41–60	61–100
Asparagus	0.98	0.96	1.00	0.99	.....	0.95	.....	.....	.....	.....	.....	.....
Peas	1.00	.....	1.07	0.98	.....	.....	0.96	.....	.....	.....	.....	.....
Snap beans	0.94	.....	0.90	.....	.....	.....	0.96	.....	.....	.....	.....	.....
Spinach	0.85	.....	.....	.....	0.79	.....	0.73	.....	.....	0.80	.....	.....
Peppers	0.96	.....	0.96	.....	.....	.....	0.95	0.95	0.97	0.96	.....	.....
Carrots	0.92	.....	.....	.....	.....	.....	0.92	.....	.....	.....	1.07	1.16
Iceberg lettuce	0.88	.....	0.84	0.97	.....	0.98	.....	.....	.....	.....	.....	.....
Tomatoes	1.11	.....	0.91	.....	0.92	.....	0.97	.....	.....	1.02	.....	.....
Cucumbers	.....	0.97	.....	.....	.....	0.98	.....	.....	0.88	.....	.....	.....
Potatoes	0.45	.....	.....	.....	.....	.....	0.58	.....	.....	.....	0.56	0.66

was noticed, but even after 123 days of storage at 0.5° C. the writer found a respiratory quotient of only 0.78. Most surprising is the fact that such a wide ratio between oxygen consumption and carbon dioxide evolution can be maintained for a period of several months. Surely the excessively low quotients cannot be explained on the basis of differential permeability of the epidermis to carbon dioxide and oxygen. If such a difference did exist, its effect would soon be overcome by an increase in the partial pressure of carbon dioxide within the tissue. Equally unlikely is the assumption that the utilization of fats and proteins caused the abnormally low quotients. If these substances had been used one should expect a quotient of 0.7 to 0.8, values much higher than those actually found. Perhaps the most plausible explanation can be based on the theory that throughout the storage period part of the carbohydrates are oxidized incompletely to organic acids. The actual quantity of acids accumulated would not be unreasonably large. Assuming an average respiration rate of 2.5 milligrams of carbon dioxide per kilogram per hour and an average quotient of 0.6, the total quantity of oxygen consumed in excess of the corresponding amount of carbon dioxide evolved would be only 0.288 gm. per 100 gm. fresh weight in a period of 100 days. This quantity would be sufficient to form 1.2 gm. of malic acid by oxidation of sugar. The accumulation of that quantity of organic acid is not unlikely. At any rate, of all the possibilities examined, this theory appears to offer the most probable explanation. The only conclusion that can be drawn from the available data is that the respiration of potatoes at low temperatures involves not only the simple oxidation of sugars to carbon dioxide and water, but also metabolic processes of greater complexity.

Low temperature injury and respiration

Low temperature injury or surface pitting is a physiological disorder which occurs in many fruits and vegetables after exposure to temperatures slightly above the freezing point for a period of one week or longer. Since the first investigation of this type of injury by Nelson (**13**), it has been suggested that surface pitting is associated with an abnormal course of respiration. Nelson demonstrated that the same kind of injury can be produced in some vegetables at room temperature by reducing the oxygen supply of the surrounding air. From the results of his experiments he concluded that the ultimate cause of pitting is the inability of the tissue to obtain or utilize sufficient oxygen for normal respiration when held at low temperatures. If this theory is correct, one should expect marked deviations in the respiratory quotient at 0.5° C. in those products which ordinarily exhibit this type of physiological disorder. Of the vegetables included in these experiments, cucumbers, peppers, and snap beans developed typical symptoms of pitting at 0.5° C. after periods varying from 10 to 20 days. Still, the data in table V suggest no unusual behavior during respiration at that

temperature. The calculated quotient always remains below unity, showing that actually more oxygen is consumed than would be required for the normal oxidation of sugars to carbon dioxide and water. Comparisons of the respiratory quotients with those observed at higher temperatures or with the quotients of other vegetables showing no injury at 0.5° C., fail to show any relationship which would suggest an abnormal course of respiration. While it must be admitted that these data do not furnish conclusive evidence to disprove the theory of NELSON, they render it extremely doubtful that suboxidation is the direct cause of low temperature injury.

Summary

Wide variations were found in the respiration rate of 10 different vegetables. For example, at 24° C. the initial respiration rate of Asparagus was found to be 59 times as high as that of potatoes.

No correlation could be found between respiration rates of different vegetables and their glucose content or carbohydrate reserve. The type of tissue, whether actively growing at time of harvest, or whether representing a storage organ, appeared to play a dominant rôle in determining the respiration rate of vegetables held under identical conditions.

In general, the respiration rate declined gradually with time at all temperatures. This decline may be attributed to the effect of the law of mass action or to a general aging effect.

Carbon dioxide production of Asparagus at 24° C. exceeded corresponding sugar losses as determined by chemical analysis of comparable samples. The results suggest that protein is decomposed and utilized in respiration to a considerable extent.

Temperature coefficients were calculated for two temperature ranges, 0.5° to 10°, and 10° to 24° C. The corresponding Q_{10} values varied from 1.6 to 5.1, usually being lower in the upper temperature range.

Respiratory quotients in general were highest soon after harvest, and with few exceptions smaller values were found at the lower temperature levels.

Spinach and potatoes gave unusually low respiratory quotients, especially at 0.5° C. As a possible explanation, it is suggested that in these vegetables, carbohydrates are converted to organic acids.

Vegetables which developed low temperature injury at 0.5° C. failed to show any unusual respiratory quotients. Based on these data, it appears unlikely that low temperature injury is the result of suboxidation.

CORNELL UNIVERSITY
ITHACA, NEW YORK

LITERATURE CITED

1. APPLEMAN, C. O., and SMITH, C. L. Effect of previous cold storage on

the respiration of vegetables at higher temperatures. Jour. Agr. Res. **53**: 557–580. 1936.

2. BARKER, JOHN. Analytic studies in plant respiration. VI. The relation of the respiration of potatoes to the concentration of sugars and to the accumulation of a depressant at low temperatures. 3. The relation of the respiration to the concentration of sucrose. Proc. Roy. Soc. (London) **119** B: 453–473. 1936.
3. BENNETT, J. P., and BARTHOLOMEW, E. T. The respiration of potato tubers in relation to the occurrence of blackheart. California Agr. Exp. Sta. Tech. Paper 14. 1924.
4. BENOY, MARJORIE P. The respiration factor in the deterioration of fresh vegetables at room temperature. Jour. Agr. Res. **39**: 75–80. 1929.
5. BISSON, C. S., JONES, H. A., and ROBBINS, W. W. Factors influencing the quality of fresh asparagus after it is harvested. California Agr. Exp. Sta. Bull. 410. 1936.
6. CHIBNALL, ALBERT CHARLES. Protein metabolism in the plant. Yale University Press. New Haven, Conn. 1939.
7. GERHART, J. Respiration of strawberry fruits. Bot. Gaz. **89**: 40–66. 1930.
8. GORE, H. C. Studies on fruit respiration. U.S.D.A. Bur. Chem. Bull. 142. 1911.
9. HALLER, M. H., and ROSE, D. H. Apparatus for determination of CO_2 and O_2 of respiration. Science, n.s. **75**: 439–440. 1932.
10. HOPKINS, E. F. Relation of low temperatures to respiration and carbohydrate changes in potato tubers. Bot. Gaz. **78**: 311–325. 1924.
11. KOHMAN, E. F. Organic acids and acid base relationship: Oxalic acid in foods. Jour. Amer. Dietet. A.S. **10**: 100–106. 1934.
12. MAGNESS, J. R., and DIEHL, H. C. Physiological studies on apples in storage. Jour. Agr. Res. **27**: 1–38. 1924.
13. NELSON, R. Storage and transportational diseases of vegetables due to suboxidation. Michigan Agr. Exp. Sta. Tech. Bull. 81. 1926.
14. PARKER, M. W., and STUART, NEIL W. Changes in the chemical composition of green snap beans after harvest. Maryland Agr. Exp. Sta. Bull. 383. 1935.
15. PLATENIUS, HANS. Effect of temperature on the rate of deterioration of fresh vegetables. Jour. Agr. Res. **59**: 41–58. 1939.
16. VICKERY, HUBERT BRADFORD, PUCHER, GEORGE W., WAKEMAN, ALFRED J., and LEAVENWORTH, CHARLES S. Chemical investigations of the rhubarb plant. Connecticut Agr. Exp. Sta. Bull. 424. 1939.
17. YEMM, EDMUND W. Respiration of barley plants. III. Protein catabolism in starving leaves. Proc. Roy. Soc. (London) **123** B: 243–273. 1937.

ANALYSIS OF PLANT EXTRACTS FOR CHLOROPHYLLS *a* AND *b* BY A PHOTOELECTRIC SPECTROPHOTOMETRIC METHOD[1]

C. L. Comar and F. P. Zscheile

(WITH ONE FIGURE)

A short note (**1**) has recently outlined the successful application of spectroscopic methods to the analysis of simple plant extracts for chlorophylls *a* and *b*. It is the purpose of this paper to present a more detailed discussion of the method and to give further results obtained on leaves from various plant sources.

Experimentation

Photoelectric spectrophotometric analysis

To analyze the chlorophyll system spectroscopically the absorption spectra of the pure standard components must necessarily be identical with those of the components as measured in the unknown solution. This implies that the state of the components be the same in the standard as in the unknown solutions. Thus, to avoid discrepancies, the same solvent must be used for both solutions.

It is also essential that all measurements be made under similar instrumental conditions. This is particularly important in the case of chlorophylls due to the narrow maxima and detailed structure of the absorption curves. The determination of the absorption coefficient of chlorophyll *a* at 6600 Å is quite sensitive to stray radiation since this value is high relative to the average absorption of the entire spectrum as limited by the region of photocell response. The absorption coefficients reported here were determined under conditions of negligible stray radiation; therefore these absorption constants can be successfully applied only to measurements made under such conditions.

The absorption coefficient obtained may also be influenced by the width of the spectral region isolated by the monochromator [see (**2**) for definition]. Analytical values for a representative plant extract remained constant under the following slit conditions:

6600 Å	slits	0.02–0.06 mm.	Spectral region	10–30	Å
6425 Å	"	0.04–0.06 mm.	"	20–30	Å
6130 Å	"	0.03–0.15 mm.	"	13–65	Å
6000 Å	"	0.06–0.14 mm.	"	25–60	Å
5810 Å	"	0.03–0.40 mm.	"	12–150	Å
5680 Å	"	0.06–0.15 mm.	"	22–55	Å

[1] Herman Frasch Foundation for Research in Agricultural Chemistry, Paper no. 214. Presented before a joint meeting of the Division of Biological Chemistry and the Division of Agricultural and Food Chemistry at the 101st meeting of the American Chemical Society, St. Louis, Missouri, April, 1941.

Errors are introduced if spectral regions are used which exceed the limits listed above.

The absorption spectra of chlorophylls *a* and *b* in ether solution, as recently determined on preparations of high purity by ZSCHEILE and COMAR (**5**), are presented in figure 1. Beer's law is used in the form:

$$a = \frac{\log_{10} \frac{I_o}{I}}{cl} \qquad [1]$$

where a = specific absorption coefficient (liters per gram centimeter)
c = concentration (grams per liter)
l = thickness of solution layer (centimeters)
I_o = intensity of radiant energy transmitted by the solvent-filled cell
I = intensity of radiant energy transmitted by the solution-filled cell

In the consideration of solutions in which n components contribute to the absorption at the wavelengths employed, the values of $\log_{10}\frac{I_o}{I}$ and of acl for the individual components are additive. The absorption coefficient a is an intensive property and is not additive. Thus, for a given wavelength,

$$\left[\log_{10}\frac{I_o}{I}\right]_{\text{observed}} = \left(\log_{10}\frac{I_o}{I}\right)_1 + \left(\log_{10}\frac{I_o}{I}\right)_2 \cdots + \left(\log_{10}\frac{I_o}{I}\right)_n = a_1c_1l_1 + a_2c_2l_2 \cdots + a_nc_nl_n \qquad [2]$$

Values for the concentration of each component may be obtained from absorption measurements at n wavelengths, where no two absorption curves coincide or intersect; however, no two wavelengths may be used at which absorption values for two components are in the same proportion. This is the general case.

For the two-component system considered here experimentally, where l remains constant, the equations are simplified as follows:

$$\text{at wavelength } \lambda' \cdots \log_{10}\frac{I_o}{I} = (a'_1c_1 + a'_2c_2)l \qquad [3]$$

$$\text{at wavelength } \lambda'' \ldots \log_{10}\frac{I_o}{I} = (a''_1c_1 + a''_2c_2)l \qquad [4]$$

where $\log_{10}\frac{I_o}{I}$ = absorption measurement at the given wavelength
a'_1 and a'_2 = specific absorption coefficients of components 1 and 2 respectively, at wavelength λ'
a''_1 and a''_2 = specific absorption coefficients of components 1 and 2 respectively, at wavelength λ''
c_1 and c_2 = concentrations of components 1 and 2 respectively, in grams per liter.

These two equations may be solved simultaneously to obtain values of the two unknowns, c_1 and c_2.

The total concentration C, in grams per liter, can be obtained also from determination of $\log_{10}\frac{I_o}{I}$ at a coincident point. In this instance the general equation [2] is reduced to:

$$\log_{10}\frac{I_o}{I} = \alpha Cl \qquad [5]$$

$$\text{where } C = c_1 + c_2 \qquad [6]$$

If the total concentration is known, the composition may be determined by measurement of the absorption at one suitable non-coincident point. Such a solution would involve the use of two simultaneous equations [2] and [6].

The analytical absorption coefficients for the chlorophyll components in ether solution which are used in this work are presented in table I. These values were determined with high precision and are the weighted averages of six different preparations in the case of chlorophyll *a* and three in the case of *b*. A discussion of the factors involved in the preparation of the pure components and the method of measurement is to be found in a previous publication (**5**).

TABLE I

ABSORPTION CONSTANTS EMPLOYED IN ANALYSIS

WAVELENGTH	SPECIFIC ABSORPTION COEFFICIENTS (FOR ETHER SOLUTIONS)	
	CHLOROPHYLL *a*	CHLOROPHYLL *b*
Å		
6600	102.	4.50
6425	16.3	57.5
6000	9.95	9.95
5810	8.05	8.05
5680	7.11	7.11
6130	15.6	8.05
5890	5.90	10.3
5460	3.03	6.24

Actually, a great many wavelengths could be used to solve this system, but for practical purposes we have chosen only a few representative points which are most advantageously applied. As a result of the nature of the curves some wavelengths are unsuitable for analytical application, and it would be impracticable and unnecessary to determine the absorption coefficients at all wavelengths with the same care and precision used for the few analytical points.

It can be shown that maximum analytical accuracy is possible where two simultaneous equations are used if the relative heights of the absorption curves at the two wavelengths are reversed for the two components. Inac-

curacies are easily introduced when readings are taken on steep slopes. In general, the accuracy is improved with an increase in the distance between the two curves at the wavelength employed. For instance, if a solution

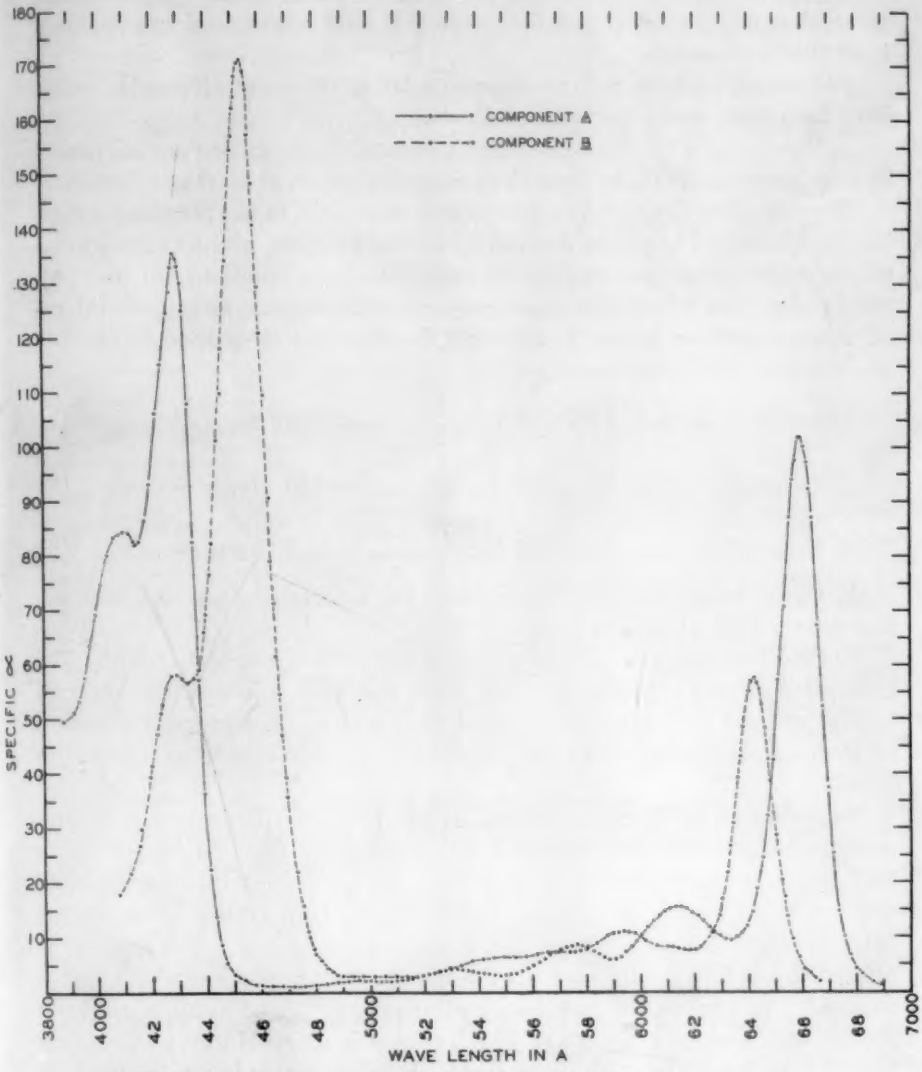


FIG. 1. Absorption spectra of chlorophylls *a* and *b* in ether solution.

containing 50 per cent. chlorophyll *a* is considered and if there were an error of +1.0 alpha unit in the absorption at 6600 Å, the apparent value would be 51.1 per cent. *a*. At 5460 Å, however, the same absolute increase

in the absorption would give an apparent value of 81.4 per cent. *a*. This means that in regions such as 5460 and 5890 Å the analytical results are extremely sensitive to light-absorbing impurities. In the case of a coincident point an error in the absorption measurement causes a percentage error of equal magnitude in the value for total concentration calculated from that wavelength.

For reasons which will be apparent later, representative calculations have been made and reported as follows:

a. Values for total chlorophyll and percentage composition are calculated by simultaneous equations from the absorption values at 6600 and 6425 Å.

The following simplified equations are convenient to use for this particular calculation. They were derived by letting $l = 1$ cm. and by substitution of the proper absorption coefficients (see table I) in equations [3] and [4], which were then solved simultaneously. It is convenient to express chlorophyll concentration in mg./l., although the standard absorption coefficients are based on concentrations in gm./l.

$$\text{Chlorophyll } a \text{ (mg./l.)} = 9.93 \log_{10}\frac{I_o}{I}_{(6600)} - 0.777 \log_{10}\frac{I_o}{I}_{(6425)} \quad [7]$$

$$\text{Chlorophyll } b \text{ (mg./l.)} = 17.6 \log_{10}\frac{I_o}{I}_{(6425)} - 2.81 \log_{10}\frac{I_o}{I}_{(6600)} \quad [8]$$

$$\text{Total chlorophyll (mg./l.)} = 7.12 \log_{10}\frac{I_o}{I}_{(6600)} + 16.8 \log_{10}\frac{I_o}{I}_{(6425)} \quad [9]$$

b. Check values for total chlorophyll are calculated from the intersection points 6000, 5810, and 5680Å.

c. Check values for percentage composition are calculated using the absorption at each of the points 6130, 5890, and 5460 Å in combination with a value of total concentration obtained from a or b. The average of values of total concentration which are unaffected by light-absorbing impurities is used.

PREPARATION OF PLANT EXTRACT.—It has been found convenient to prepare the plant extract as follows: A weighed sample (2 to 10 gm.) of fresh leaf tissue is disintegrated in a Waring Blendor containing acetone to which a small amount of calcium carbonate has been added. The ease of extraction depends on the type, age, and condition of leaf. The mixture is filtered through paper by suction; the residue may be washed with ether if necessary, to remove the last traces of pigment. The filtrate is made to 500 ml. with acetone and a 25- or 50-ml. aliquot is pipetted into a separatory funnel containing about 50 ml. of ether. Distilled water is carefully added and the fat-soluble pigments are transferred quantitatively into the ether layer. The ether is then washed exhaustively by scrubbing through distilled water by the method first suggested by MACKINNEY (**3**). At present we wash the solutions at least twenty times. The solution is then made to

100 ml. with ether and dried over anhydrous sodium sulphate. When optically clear it is ready for spectroscopic measurement.

ANALYTICAL RESULTS.—Since carotenoids are generally present in the plant extract it was necessary to investigate the effect of these pigments on the analytical results. Wavelengths below 5460 Å have not been used due to carotenoid interference.

TABLE II

EFFECT OF CAROTENE ON THE ANALYSIS OF CHLOROPHYLL FROM SPINACH

WAVELENGTH	SAMPLE 1	SAMPLE 2	SAMPLE 3
	CHLOROPHYLL ($a+b$) (CAROTENOIDS REMOVED)	CHLOROPHYLL ($a+b$) (2 MG. BETA-CAROTENE PER 100 ML.)	CHLOROPHYLL ($a+b$) (6.2 MG. BETA-CAROTENE PER 100 ML.)
Å			
6600	80.3% a	79.4% a	79.2% a
6425	60.6 mg./l.*	59.0 mg./l.	59.7 mg./l.
6000	60.0 mg./l.	60.4 mg./l.	62.3 mg./l.
5810	60.2 mg./l.	60.3 mg./l.	62.2 mg./l.
5680	61.0 mg./l.	61.8 mg./l.	66.5 mg./l.
6130	79.8% a	80.1% a	83.4% a
5890	78.9% a	77.3% a	72.7% a
5460	78.2% a	64.8% a	38.6% a

* Concentration values in all tables, unless otherwise designated, represent the concentration of total chlorophyll ($a+b$) expressed as mg. per liter in the measured solution.

Table II shows the agreement among results obtained by use of various wavelengths when carotenoids are absent, and the effects produced when known amounts of carotene are added. Sample 1 represents a solution of chlorophyll ($a+b$) from which all carotenoids had been removed. This was done by washing with 80 per cent. methanol and subsequent precipitation of the chlorophylls from the petroleum ether solution; the chlorophylls were precipitated on a sucrose layer and washed exhaustively with petroleum ether to remove carotenoids (**5**).

In this case the a to b relation does not represent that present in the original leaf tissue due to the preferential removal of chlorophyll b by the methanol. Samples 2 and 3 were aliquots of sample 1 to which pure beta-carotene was added as indicated. This sample[2] of carotene had been obtained from a commercial preparation and had been recrystallized five times. Sample 2 had approximately the same chlorophyll-carotenoid ratio as found in the usual spinach leaf.

Table III represents the data of table II corrected by calculation for the carotene contribution to the absorption. This was done by use of the

[2] This carotene was made available to us through the courtesy of J. W. WHITE, JR., and B. W. BEADLE of this laboratory.

TABLE III

ANALYSIS OF CHLOROPHYLL FROM SPINACH
(CORRECTED FOR CAROTENE CONTENT BY CALCULATION)

WAVELENGTH	SPECIFIC ABSORPTION COEFFICIENTS OF beta-CAROTENE IN ETHER SOLUTION	SAMPLE 1 CHLOROPHYLL ($a+b$)	SAMPLE 2 CHLOROPHYLL ($a+b$) (+2 MG. beta-CAROTENE PER 100 ML.)	SAMPLE 3 CHLOROPHYLL ($a+b$) (+6.2 MG. beta-CAROTENE PER 100 ML.)
Å				
6600	0.06	80.3% *a*	79.4% *a*	79.2% *a*
6425	0.09	60.6 mg./l.	59.0 mg./l.	59.7 mg./l.
6000	0.22	60.0 mg./l.	60.0 mg./l.	60.9 mg./l.
5810	0.41	60.2 mg./l.	59.2 mg./l.	59.0 mg./l.
5680	0.60	61.0 mg./l.	60.0 mg./l.	61.1 mg./l.
6130	0.16	79.8% *a*	79.5% *a*	81.0% *a*
5890	0.33	78.9% *a*	80.5% *a*	80.0% *a*
5460	1.32	78.2% *a*	79.5% *a*	81.0% *a*

known amount added and the specific absorption coefficients for beta-carotene as determined in ether at the given wavelengths. The coefficients reported for beta-carotene are the result of a single series of measurements and are not to be considered as standard values.

It is possible to account quantitatively for pure chlorophyll *a* which has been added to the ether solution from a plant extract. The concentration of the chlorophyll *a* had been determined by evaporation of an aliquot of the solution to dryness (**5**). Table IV presents the type of data obtained:

It is evident that agreement between columns 3 and 4 is excellent, showing that the absorption properties of the pure chlorophyll *a* remain unchanged after addition to the ether solution from the extract.

TABLE IV

RECOVERY OF CHLOROPHYLL *a* ADDED TO EXTRACT FROM SPINACH

	1	2	3	4
WAVELENGTH	$\log_{10}\frac{I_o}{I}$ EXTRACT	$\log_{10}\frac{I_o}{I}$ EXTRACT + CHLOROPHYLL *a*	$\log_{10}\frac{I_o}{I}$ CHLOROPHYLL *a* (2–1)	$\log_{10}\frac{I_o}{I}$ CHLOROPHYLL *a* (CALCULATED FROM KNOWN AMOUNT ADDED
Å				
6130	0.386	0.526	0.140	0.140
6000	0.299	0.393	0.094	0.090
5810	0.231	0.304	0.073	0.072
5680	0.208	0.271	0.063	0.064

TABLE V

ANALYSIS FOR TOTAL CHLOROPHYLL AND PERCENTAGE COMPOSITION

WAVELENGTH	SPINACH	TOMATO	BARLEY	RYE	OATS	WHEAT	ALFALFA	CORN	TOBACCO	BROCCOLI TIPS	COCKLE-BUR	SWEET POTATO
Å												
6600	71.1	71.0	72.8	73.6	73.3	74.3	74.6	77.2	70.6	70.0	70.3	68.1% *a*
6425	41.8*	57.6	59.7	63.3	34.0	63.2	65.1	43.7	55.3	50.0	35.0	50.0 mg./l.
6000	42.2	57.9	61.0	66.3	35.7	64.9	66.3	43.7	58.7	55.5	37.2	51.0 mg./l.
5810	41.8	57.7	59.7	66.4	36.4	65.2	66.7	44.6	60.2	49.5	36.2	51.0 mg./l.
5680	41.4	56.3	58.7	65.5	35.4	64.2	64.5	43.3	58.3	50.5	36.0	51.2 mg./l.
6130	70.4	70.7	73.4	75.2	74.8	75.5	79.1	76.3	70.3	82.2	74.0	68.2% *a*
5890	66.6	68.2	66.4	64.1	62.0	65.0	63.4	70.0	59.1	58.2	63.6	62.5%
5460	64.5	65.1	59.8	50.4	54.2	56.1	51.7	64.8	59.7	44.1	59.2	57.9%
Total chlorophyll mg./gm. fresh leaf	.....	.....	.....	1.62	2.22	2.97	2.40	.....	1.25	.....	1.93	.....

* See footnote of table II.

Table V presents typical analytical values obtained for leaves of several plants.

The overall reproducibility of the analytical method is very good, as shown by the following typical experiment. Symmetrical sweet potato leaves were cut lengthwise in half to yield two similar samples which were analyzed with the following results:

Sample 1 ..... 2.28 mg. total chlorophyll per gm. fresh leaf
69.6% chlorophyll *a*

Sample 2 ..... 2.38 mg. total chlorophyll per gm. fresh leaf
69.4% chlorophyll *a*

STORAGE OF SAMPLES.—The final ether solution from spinach leaves, after removal from the sodium sulphate layer, may be stored for at least three months without change of analytical results. Table VI shows the effect of storage on the analytical values. The extract was prepared from fresh spinach leaves and the samples stored in the dark at about 5° C. The values were determined from absorption measurements at 6600 and 6425 Å. Similar tests on other sources have not been tried.

TABLE VI

EFFECT OF STORAGE ON CHLOROPHYLL ANALYSIS OF ETHER SOLUTIONS FROM SPINACH EXTRACTS

DATE ANALYZED	CHLOROPHYLL ($a+b$)	CHLOROPHYLL *a*
Jan. 17, 1941	9.85 mg./l.	71.7%
Jan. 20, 1941	9.76	70.9
Feb. 10, 1941	9.76	70.8
Feb. 25, 1941	9.82	72.1
Mar. 15, 1941	9.87	71.6
April 30, 1941	9.98	70.8

TABLE VII shows the effect of storage of the leaf material on the analytical values. Leaves from corn seedlings were treated as follows:

a. Stored fresh under nitrogen at −20° C.

b. Dried in vacuum at 40° C. and stored under nitrogen at −20° C.

The analyses were made with absorption values at wavelengths 6600 and 6425 Å.

The low value for total chlorophyll in the case of the last dried sample is attributed to obviously incomplete extraction.

Discussion

As may be noted from the above data, the largest source of error appears to be due to the uncertainty of complete extraction. Usually 10 to 15 minutes in the Blendor is sufficient for complete extraction of young fresh leaves. Older fresh leaves and dried material often require a second ex-

TABLE VII

EFFECT OF STORAGE ON CHLOROPHYLL ANALYSIS OF LEAVES FROM CORN

DATE ANALYZED	STORAGE CONDITIONS	PERCENTAGE CHLOROPHYLL *a*	TOTAL CHLOROPHYLL MG./GM. FRESH LEAF
		%	*mg.*
Mar. 27, 1941	original sample	74.6	1.81
April 3, 1941	fresh	76.6	1.78
	dry	76.4	1.72
April 28, 1941	fresh	77.6	1.86
	dry	75.0	1.34

traction, and even then the removal of pigment is not always complete. Dried leaves are particularly difficult to extract satisfactorily.

Acetone must be removed from the ether solution as completely as possible. This is done by scrubbing through distilled water. Experiments with solutions of pure chlorophyll *a* have shown that scrubbing the solutions about twenty times, as described, will reduce the acetone concentration of the ether solution enough to avoid any error due to the presence of the acetone. No critical study has been made to determine the minimum amount of washing necessary.

du Pont's technical grade of diethyl ether, after purification with solid NaOH and distillation, was used in this work and found to be satisfactory. It seems likely that uncertainties may be introduced by using ether which contains ethanol.

The final solutions of the original standard components were prepared by washing the ether solution with water and drying over anhydrous sodium sulphate (**5**). In order that unknown solutions be comparable with the standards, the former are washed, made to volume, and dried over sodium sulphate. Finely powdered sodium sulphate will delay clarification of the solutions and should not be used. We have found that the sodium sulphate does not adsorb any pigment from the solution during the first 24 hours. After 48 hours, however, a significant decrease in the pigment concentration occurs. Therefore, if the final extract is to be stored for longer periods of time, it should be removed from the sodium sulphate layer.

The values obtained for sample 1 in table II indicate the identity of spectra of the standard components with those of the components in the plant extract. The agreement between wavelengths is quite good especially when the extreme sensitivity of values calculated from 5890 and 5460 Å is considered. The effect of carotenoids is most evident at wavelengths 5460 and 5890 Å, as is shown in sample 2 by the apparent decrease in percentage of chlorophyll *a*. In sample 3, which contained an excessive amount of carotene, only values calculated from 6600 and 6425 Å were unaffected. At

6130 Å the apparent value of percentage of chlorophyll *a* is increased by the presence of light-absorbing impurities because at this wavelength the chlorophyll *a* absorption curve is higher than that of *b*. That these effects are actually due to the presence of carotene is confirmed by the agreement shown in table III.

The data presented in table V indicate that the method is, in general, reliable for most of the plants listed except for values calculated from measurements at 5460 and 5890 Å. These deviations are similar to those produced by the addition of beta-carotene to extracts from which all carotenoids had been removed. It seems evident, therefore, that these deviations are attributable chiefly if not entirely to the presence of carotenoids. In the cases of alfalfa, broccoli, and cocklebur there is evidence of the presence of impurities absorbing at 6130 Å as shown by the apparent increased percentage of chlorophyll *a* at this wavelength; this may also be due to the presence of carotenoids. In the case of two samples of broccoli tips, the values calculated from 6000 and 6130 Å were unusually high, which indicates increased absorption in this region.

Values for the amount of chlorophyll per gram of fresh leaf are included merely to give an order of magnitude. Before comparative values could be listed it would be necessary to consider such factors as age of leaf, position of leaf, and moisture content. In the cases studied, the chlorophyll content varied from 1.2 to 3.0 mg. per gm. fresh leaf. It should be noted that the rye leaves were taken from relatively old plants and that the tobacco leaves were taken from plants which appeared etiolated.

The values of percentage composition and total chlorophyll presented here agree quite well with the original analyses of WILLSTÄTTER and STOLL (**4**).

It may be noted from figure 1 that the curves of chlorophyll *a* and *b* intersect also at 6265 Å. In every plant extract examined by us the value for total chlorophyll as calculated from this wavelength was 5 to 6 per cent. higher than values from other wavelengths. Repeated experiments indicated that this difference was not due to instrumental error or errors in the standard absorption coefficients. The substance causing absorption at this wavelength is not separated from the chlorophylls by precipitation and washing with petroleum ether but is removed from the chlorophylls in the adsorption part of the purification process.

The details of the method were developed specifically for spinach and corn leaves. There is excellent agreement between wavelengths (except 5460 and 5890 Å) for these two cases. Similar agreement is obtained for tomato, barley, and sweet potato leaves. In the other cases, however, the checks are not as good. It may be that this could be improved by slight modifications of the method for the plant in question.

For routine analysis it is convenient to use measurements only at wavelengths 6600 and 6425 Å. In cases such as tomato and barley in which agreement between determinations from the several wavelengths is similar to that of spinach, one would be justified in using wavelengths 6600 and 6425 Å for routine analysis. For such cases as tobacco and broccoli, in which determinations at other wavelengths do not check those obtained from 6600 and 6425 Å, the necessity for further work is indicated to clarify the causes for such discrepancies. It is emphasized that each plant material should be studied before this method can be applied. Discrepancies will be caused by the presence in the extract of any substance other than chlorophyll which absorbs light at the wavelength employed.

Summary

A simple, rapid, and accurate spectroscopic method for the determination of chlorophylls *a* and *b* in plant materials has been described.

An acetone extract is made from fresh leaf material. The fat-soluble pigments are transferred to ether, the acetone removed, and the solution dried with sodium sulphate. Spectroscopic analysis for chlorophyll *a* and chlorophyll *b* is made at wavelengths 6600 and 6425 Å. The reliability of values obtained from other wavelengths depends considerably upon the carotenoid content.

The chemical precautions and instrumental conditions essential for quantitative results have been discussed.

The method has been applied to twelve different plant sources.

PURDUE UNIVERSITY
LAFAYETTE, INDIANA

LITERATURE CITED

1. COMAR, C. L., and ZSCHEILE, F. P. Spectroscopic analysis of plant extracts for chlorophylls *a* and *b*. Plant Physiol. **16**: 651–653. 1941.
2. HOGNESS, T. R., ZSCHEILE, F. P., JR., and SIDWELL, A. E., JR. Photoelectric spectrophotometry. An apparatus for the ultraviolet and visible spectral regions: its construction, calibration, and application to chemical problems. Jour. Phys. Chem. **41**: 379–415. 1937.
3. MACKINNEY, G. Criteria for purity of chlorophyll preparations. Jour. Biol. Chem. **132**: 91–109. 1940.
4. WILLSTÄTTER, R., and STOLL, A. Investigations on chlorophyll. English translation by SCHERTZ, F. M., and MERZ, A. R. 1928.
5. ZSCHEILE, F. P., and COMAR, C. L. Influence of preparative procedure on the purity of chlorophyll components as shown by absorption spectra. Bot. Gaz. **102**: 463–481. 1941.

SPECIFIC BIOLOGICAL ACTIVITY OF TOBACCO-MOSAIC VIRUS AS INFLUENCED BY AGE OF LESION AND NITROGEN SUPPLY

ERNEST L. SPENCER

(WITH TWO FIGURES)

Introduction

A combination of ultracentrifugation techniques with recently improved methods of biological assay has made it possible to demonstrate that nitrogen supply has a direct effect on the virus entity of tobacco mosaic *in vivo*. This effect is shown by the fact that the rate of virus multiplication in the infected plant as a whole is dependent to a large extent on the available nitrogen supply (**10**). An interruption of the nitrogen supply retards or may even inhibit further virus increase, thereby permitting the demonstration of virus inactivation within the host (**8**). Now it appears that with these techniques much more can be learned from determinations of the specific biological activity of virus preparations. The earlier studies have raised several interesting questions concerning virus behavior in living tissue. Is the rate of virus multiplication in the inoculated leaf affected by nitrogen supply? Is the virus in mature leaves of a nitrogen-deficient plant subject to proteolysis such as normal plant proteins undergo, with subsequent translocation of the proteolytic products to other parts of the plant? Does the virus possess its full biological activity as soon as it is formed, or does it go through a transition stage during which it possesses only a fraction of its potential specific activity? If such a stage does exist, what factors are effective in increasing the specific activity of the virus complex?

Experiments have been carried out in an endeavor to answer some of these questions. The results of these studies, a partial summary of which has been published (**9**), are reported in this paper.

Methods and materials

Turkish tobacco plants (*Nicotiana tabacum* L.) were used as experimental host plants and, except where otherwise stated, were grown in nutrient sand cultures. The composition of the solutions, one a complete nutrient solution and the other deficient only in nitrogen, has already been published (**8**). The virus used was that of tobacco mosaic (*Marmor tabaci* H.). At time of harvest, the portion of the plant under investigation was frozen at $-14°$ C. for 24 hours and then minced. The juice, cleared of insoluble matter and adjusted to approximately pH 7.0 with K_2HPO_4, was assayed for total nitrogen and total proteins as previously described (**7**).

Inorganic nitrogen was determined by the method proposed by SESSIONS and SHIVE (**6**), modified to permit the use of 1-ml. samples. The term *virus protein* as used in this paper refers to the proteinaceous sediment obtained by repeated ultracentrifugation. Although this material is practically homogeneous, it is not necessarily crystalline. All preparations received two treatments of 1 hour each in the ultracentrifuge as previously described (**10**). The virus-protein content of the expressed juice was calculated from the nitrogen content of the ultracentrifuged sediment suspended in 0.1 M potassium phosphate buffer at pH 7.0. The virus activity of each preparation was assayed on bean plants (*Phaseolus vulgaris* L. var. Early Golden Cluster) by a modification (**11**) of the local lesion method (**1**).

Experimentation

Recent studies (**8**) indicated that in juice expressed from nitrogen-deficient tobacco plants infected with tobacco mosaic, the amount of soluble plant proteins as well as that of virus protein remained practically constant. In mature leaves of such plants it has been established (**4, 12**) that plant proteins are hydrolyzed and the proteolytic products translocated to the growing tip where they may be resynthesized into plant proteins. The published data pertaining to the virus concentration in nitrogen-deficient plants do not show whether virus is affected by similar proteolytic changes or whether it remains for the most part at the site of formation.

VIRUS MULTIPLICATION IN NORMAL MATURE LEAVES

In order to determine whether virus is subject to normal protein hydrolysis under conditions of nitrogen starvation, a study was made of the virus multiplication in mature leaves of infected tobacco plants in various stages of nitrogen deficiency. Several hundred plants receiving the complete nutrient solution were inoculated when about 6 inches in height by rubbing one of the lower mature leaves on each plant with a virus suspension. At 5-day intervals the inoculated leaves from 12 or more representative plants were harvested, frozen, and assayed as previously described. Ten days after inoculation, when the plants were systemically infected, they were divided into two groups. One group continued to receive the complete nutrient solution, with the nitrogen content raised to 400 p.p.m.; the other group was changed to the minus-nitrogen solution. The plant-protein and virus-protein contents of the juice at each harvest, together with the plant-protein content of the juice from corresponding leaves of healthy plants on similar nutrient treatments, are recorded in table I.

As seen in table I, the amount of normal plant proteins in the juice from the inoculated leaf, calculated as the difference between the total protein content and the virus-protein content, gradually decreased even in plants

TABLE I

CONCENTRATION OF TOTAL NITROGEN AND PROTEIN IN JUICE FROM INOCULATED LEAVES AND CORRESPONDING LEAVES FROM HEALTHY PLANTS AT INTERVALS AFTER INOCULATION*

TIME OF HARVEST: DAYS AFTER INOCULATION		NUMBER OF PLANTS FROM WHICH LEAF WAS HARVESTED		AVERAGE NITROGEN CONTENT PER LEAF				AVERAGE PROTEIN CONTENT PER LEAF			
				TOTAL		INORGANIC		PLANT PROTEIN		VIRUS PROTEIN	
		+N TREATMENT	−N TREATMENT	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS
	days			*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
	0	12	.....	2.24	.....	0.81	.....	7.7	.....	.....	.....
	5	18	.....	1.37	.....	0.27	.....	4.6	.....	1.04	.....
Inoculated leaf	10	18	18	1.86	1.85	0.06	0.06	3.8	3.8	3.08	3.08
	15	18	18	1.51	1.67	0.06	0.01	2.3	1.5	4.92	6.16
	20	16	20†	2.07	1.79	0.04	0.01	2.5	1.8	7.42	6.96
	25	15†	.....	2.22	.....	0.04	.....	3.8	.....	7.42	.....
	0	12	.....	2.24	.....	0.81	.....	7.7	.....	.....	.....
Healthy control leaf	5	15	.....	1.34	.....	0.44	.....	4.9	.....	.....	.....
	10	12	12	1.08	1.08	0.20	0.20	4.2	4.2	.....	.....
	15	12	15	0.71	0.59	0.04	0.07	2.3	2.0	.....	.....
	20	15	18	0.56	0.34	0.09	0.01	1.5	0.7	.....	.....

* Minus-nitrogen treatment started on 10th day after inoculation.
† Inoculated leaves yellow and slightly shriveled.

receiving nitrogen. This decrease was obviously not due to the presence of virus in these leaves, as a similar decrease was also found in juice from corresponding leaves of healthy plants. Apparently, the nitrogen supplied in the nutrient solution was not adequate to meet the requirements of these rapidly growing plants, and therefore nitrogen in one form or another was drawn from the lower leaves to supply the growing tip. It was of interest to note that on the day of inoculation over one-third of the nitrogen in the leaf to be inoculated was present as inorganic nitrogen (nitrate- or ammonium-nitrogen). This reserve of inorganic nitrogen was nearly exhausted during the first ten days after inoculation. At all test periods, less inorganic nitrogen was found in juice from inoculated leaves than in that from corresponding leaves of healthy plants. These observations corroborate earlier findings (**8**) which indicated that virus formation utilizes simple forms of nitrogen rather than nitrogen previously synthesized into plant proteins.

As was to be expected, in the inoculated leaf of nitrogen-deficient plants the soluble plant-protein content decreased much faster than in the inoculated leaf of nitrogen-fed plants.

During this period when the soluble plant-protein content gradually decreased, the virus-protein content increased, not only in the inoculated leaf of nitrogen-fed plants but even in the inoculated leaf of plants transferred to the minus-nitrogen treatment. During the 10 days following the change in nitrogen treatment, the soluble plant-protein content in the inoculated leaf of plants on the minus-nitrogen treatment decreased about 50 per cent., whereas the virus-protein content more than doubled during the same interval. Such an observation might lead one to conclude that these soluble plant proteins were utilized in virus formation. This is not necessarily the case, as earlier observations (**8**) showed that the total soluble plant-protein content remained practically constant in the plant, indicating that the proteins disappearing from the inoculated leaf could be accounted for in other parts of the plant.

It was necessary to terminate the minus-nitrogen treatment 10 days after transfer or 20 days after inoculation, as the mature inoculated leaves of the nitrogen-deficient plants were extremely yellow and had begun to shrivel, a characteristic reaction of nitrogen starvation. It is difficult to explain the continued formation of virus protein in the inoculated leaf of plants deprived of nitrogen. The analytical data did show that the amount of simple organic nitrogenous compounds present in the juice was sufficient to allow continued virus multiplication during this limited 10-day period intervening between the initiation of the minus-nitrogen treatment and the termination of the experiment. It is also possible that, under certain conditions, the break-down products of normal proteolysis may be utilized to some extent for virus formation. There is no evidence to indicate that virus can initiate

such proteolysis, but it may take advantage of an existing physiological change.

The results in table I further substantiate earlier observations (**8**) that a nitrogen-deficient plant is unable to use the nitrogen in the virus for normal protein synthesis, since virus continued to multiply in mature leaves at the same time that plant proteins were being hydrolyzed and withdrawn from these leaves. The experimental data also show that virus protein was affected only slightly, if at all, by the normal proteolytic processes in the plant, as the virus continued to increase in leaves where these hydrolytic processes were taking place. Normal plant proteins were hydrolyzed and the decomposition products translocated, but most of the virus remained at the site of formation. It is known (**5**) that some virus does migrate to other tissues and in this way initiates systemic infection, but it is believed (**3**) that only small amounts of virus are involved in this movement.

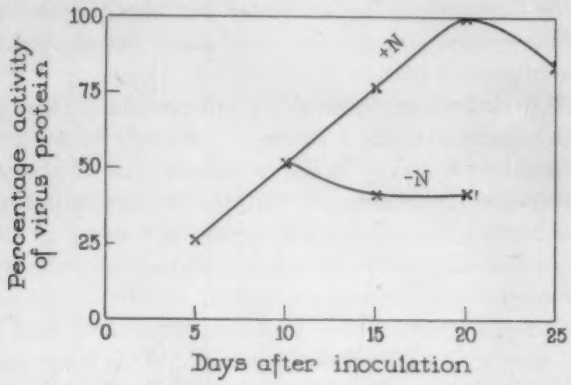


FIG. 1. Relative biological activity per unit weight of virus protein in juice from inoculated leaves at intervals after inoculation, the most active sample arbitrarily chosen as 100 per cent. Ten days after inoculation, half of the plants were changed to the minus-nitrogen treatment, as represented by the – N line.

Earlier work (**2**) had indicated that the virus activity of juice from the inoculated leaf reached a maximum within 7 to 10 days. In the present experiment, virus concentration as measured by virus-protein content not only increased beyond the 10th day but more than doubled between the 10th and 20th days. From these data alone no conclusion could be drawn regarding the specific biological activity of the virus. It was possible that after the 10th day virus might be inactivated at about the same rate that it formed, and therefore no increase in activity per ml. of juice would occur beyond this point. In order to determine whether this were true, tests were carried out to measure the relative virus activity of ultracentrifuged preparations obtained from inoculated leaves at intervals after inoculation. Activity measurements were made as soon as the final preparation, obtained 25 days

after inoculation, could be included. Meantime, suspensions of ultracentrifuged virus proteins from the earlier harvests had been stored at 4° C. in a refrigerator.

The results of these activity tests are presented graphically in figure 1. The +N line represents the activity measurements of virus from the inoculated leaves of the nitrogen-fed plants; the −N line represents activity data of inoculated leaves after the plants were deprived of an external supply of nitrogen. The data from nitrogen-deficient plants indicated that the specific activity of the virus showed no further increase following the change to the minus-nitrogen treatment but remained fairly constant.

The data from the nitrogen-fed plants show that the biological activity of virus isolated 5 days after inoculation was considerably lower than that of virus isolated at any of the later harvests. The specific activity (activity per unit weight) of virus protein 5 days after inoculation was only 25 per cent. of that of virus protein isolated 15 days later. Between the 5th and 10th days after inoculation the specific activity, not of the inoculated leaf as a whole but of the virus protein itself, about doubled and this increase continued until it reached a maximum around 20 days after inoculation.

It had been expected that the virus would be fully active as soon as it was formed. These findings were therefore wholly unexpected and indicated rather that virus isolated from lesions shortly after infection was much lower in activity per unit weight than that isolated later.

In the above experiment lower activity of samples taken soon after infection might be thought to be due to longer storage of such samples before testing. No appreciable loss of activity is believed to take place during storage for limited periods under such conditions. In subsequent tests, however, preparations compared with one another were all subjected to the same storage and other experimental conditions.

Virus multiplication in nitrogen-starved leaves

Inasmuch as the experiments discussed up to this point were carried out with plants in which the mature leaf when inoculated was amply supplied with nitrogen, the question arose as to what would happen to the virus if inoculated into a yellow, nitrogen-deficient leaf. Would virus multiplication take place and, if so, what would be the specific activity of virus formed under these conditions? A group of plants about 15 inches tall, that had received nitrogen for 10 days and then had been deprived of it for 15 days, was inoculated with a virus suspension by rubbing a mature yellow leaf of each plant. Corresponding leaves on nitrogen-fed plants were inoculated and used as controls. Since earlier tests showed that on nitrogen-deficient plants of this size the mature leaves rapidly shrivel and die, it was found advisable to remove the top of the plant by severing the stem just above the

leaf inoculated. This prevented further withdrawal of food from the inoculated leaf, and the leaf remained turgid for 10 to 12 days before it began to dry up. The nitrogen-fed plants were cut in the same way at time of inoculation. It was also found advisable to shade the plants on bright days to prevent injury. On the 5th and 10th days after inoculation, representative inoculated leaves and corresponding leaves from healthy plants, treated in the same way except for inoculation, were harvested and assayed as previously described. The experimental data are recorded in table II.

TABLE II

TOTAL AMOUNT AND SPECIFIC ACTIVITY OF VIRUS PROTEIN FROM NORMAL INOCULATED LEAF AND FROM LEAF INOCULATED WHILE IN A SEVERELY NITROGEN-DEFICIENT CONDITION*

TIME OF HARVEST: DAYS AFTER INOCULATION		PROTEIN CONTENT PER LEAF				SPECIFIC ACTIVITY† OF VIRUS PROTEIN	
		PLANT PROTEINS		VIRUS PROTEIN			
		N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS
	days	*mg.*	*mg.*	*mg.*	*mg.*	%	%
Inoculated leaf	0	4.3	1.5	.....	.....	.....	.....
	5	10.3	2.4	1.5	0.5	35	10
	10	16.5	1.7	9.8	1.1	100	38
Healthy leaf	0	4.3	1.5	.....	.....	.....	.....
	5	12.2	2.4	.....	.....	.....	.....
	10	14.1	3.4	.....	.....	.....	.....

* The portion of the plant above the inoculated leaf and above the corresponding leaf on the healthy plants was removed at time of inoculation.

† Biological activity per unit weight of virus protein relative to that of virus from inoculated leaf of N-fed plants 10 days after inoculation.

The amount of plant proteins in juice from both the inoculated leaf and the corresponding healthy leaf on plus-nitrogen treatment increased nearly 400 per cent. during the 10-day experimental period. During this period the individual leaves about doubled in size, as measured by green weight and volume of juice expressed after freezing. The plant proteins in the juice from the inoculated nitrogen-deficient leaves did not change appreciably in concentration, but in the healthy nitrogen-deficient leaves they increased over 200 per cent. This increase seemed rather surprising since no external nitrogen was available, but apparently proteins or proteolytic decomposition products in the roots were translocated, not to the top since this had been removed, but up as far as the one remaining leaf.

The data on the virus-protein content of the inoculated leaves show that some virus multiplication did take place in the nitrogen-deficient leaf but that it was greatly limited. Five days after inoculation, the virus-protein

content of nitrogen-fed leaves was 3 times as great as that of the nitrogen-deficient leaves; five days later, or 10 days after inoculation, the virus content of these nitrogen-fed leaves was 9 times as great. This indicated a more rapid rate of virus multiplication in the nitrogen-fed leaves than in the nitrogen-starved leaves. The virus activity measurements show that the specific biological activity of the virus in the nitrogen-fed leaves increased over 250 per cent. faster than did the virus in the nitrogen-deficient leaves during the first 10 days after inoculation. This experiment shows that, although the virus multiplied to some extent in the nitrogen-starved leaves, the biological activity per unit weight of this virus protein was considerably less than that displayed by virus formed under conditions where nitrogen was not a limiting factor. These results indicate that nitrogen may be an important factor in determining the specific biological activity attained by the virus following inoculation into living tissues.

Specific activity of virus from inoculated leaf as compared with that of virus from top of the plant

In view of the surprising results of the first experiment showing that virus in young lesions displays only a fraction of its potential specific activity, another experiment was carried out to determine whether the specific activity of virus isolated from the inoculated leaf differs from that isolated from the remainder of the plant at the same time. The plants were inoculated and treated as in the first experiment. Data from this experiment are expressed graphically in figure 2, figure 2A representing the nitrogen-fed plants, and figure 2B the plants deprived of nitrogen 10 days after inoculation. In both graphs, the activity of the virus in the inoculated leaf at each test period is designated as 100 per cent. In the nitrogen-fed plants, virus from the inoculated leaf was more active than that from the top of the plant for the first 10 days after inoculation. By the 15th day virus from the two sources was about equal in specific activity and continued to be so for the remainder of the experiment.

Since it can be assumed that some of the lesions in the inoculated leaf were formed earlier than any of those in the top of the plant and were therefore somewhat older, these data corroborate those in figure 1, which indicated that virus in young lesions was less active than that in older lesions, provided no inactivation had taken place. The relationship shown by the data in figure 2B for the nitrogen-deficient plants is similar to that shown by the nitrogen-fed plants except that the differences on the 15th and 20th days after inoculation are more striking. Part of this greater difference in the nitrogen-deficient plants may be due to a partial inactivation of the virus in the inoculated leaf, as shown in figure 1. Any virus inactivation due to nitrogen deficiency, a phenomenon previously reported

(8), would be expected to take place in the inoculated leaf earlier than in the top of the plant, since nitrogen starvation is more acute in the lower mature leaves. Moreover, a part of the difference observed in figure 2B may be due directly to the influence of nitrogen on virus multiplication. Since it has been shown that in a nitrogen-deficient plant most of the nitrogen is concentrated near the top of the plant, the specific activity of virus in this region might be expected to exceed that of virus located in regions such as the inoculated leaf where nitrogen is absent or present in small amounts.

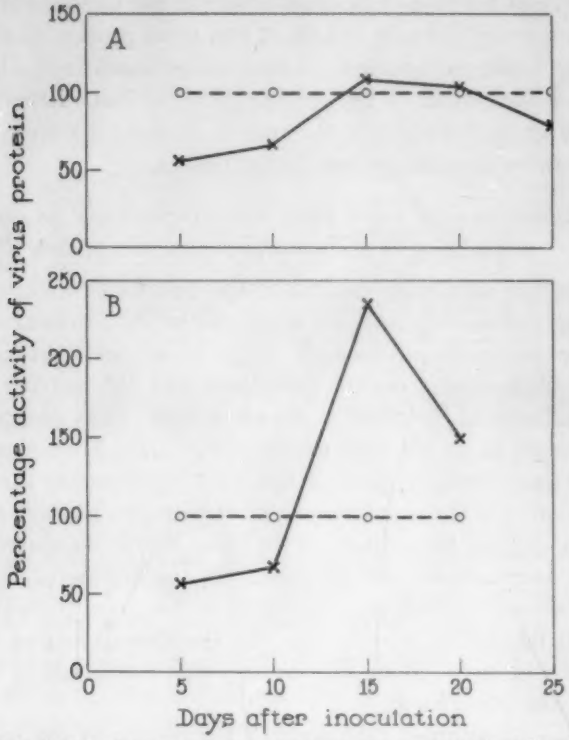


FIG. 2. Biological activity per unit weight of virus protein in juice from top of the plant (solid line) relative to that from inoculated leaf (broken line) at intervals after inoculation. A, nitrogen-fed plants; B, plants deprived of nitrogen 10 days after inoculation. The activity of the virus from the inoculated leaf at each harvest was arbitrarily designated as 100 per cent.

If this supposition is true, the changes in activity of the virus in the top of the plant would tend to be like those shown in figure 1 by the +N line, whereas the changes in the activity of virus in the inoculated leaf would be represented by the −N line.

It is difficult to comprehend the full significance of these results at the present time. It has been assumed by most workers with plant viruses

that the biological activity of the virus is at its maximum as soon as the virus is formed, and any increase in activity is due to a multiplication of these infectious units. The results observed in these experiments suggest that at first the virus expresses only a portion of its potential activity and that its specific activity becomes greater and greater with time. The importance of nitrogen in this change, as has already been pointed out, may be inferred from the data on the nitrogen-deficient plants. As shown by the data in table I, virus-protein content increased at about the same rate in the inoculated leaf of the nitrogen-deficient plant as it did in that of a nitrogen-fed plant. The graph in figure 1, however, shows that increase in specific activity of the virus took place only as long as nitrogen was supplied. Soon after nitrogen was withheld, no further increase in virus activity could be demonstrated. A similar inference can be drawn from the data in figure 2B.

Further ultracentrifugation studies

Further experiments were carried out to determine whether any differences other than specific biological activity could be detected between virus from recently infected leaves and virus from leaves infected for a longer period. Although the results are preliminary, it was deemed advisable to include them in the present paper. Tobacco plants about 6 inches tall, grown in composted soil, were inoculated by rubbing one mature leaf on each plant. Fifteen days later a second group of young plants was inoculated in the same way. Five days after inoculation of the second group the inoculated leaves from both groups of plants were harvested and the virus isolated by ultracentrifugation as previously described. This procedure subjected both virus preparations to the same experimental and environmental conditions. Activity tests on these two preparations showed that virus from the older lesions was 37 per cent. more active than that from leaves recently infected. This difference in activity is not as great as that obtained in experiments reported earlier in this paper. The smaller difference may be due to the fact that these plants were grown in soil, whereas those that gave large differences in the previous experiments were all grown in nutrient sand cultures with an abundant nitrogen supply.

On the day following isolation, the virus preparations from the two groups of plants were analyzed in the analytical ultracentrifuge to determine their rate of sedimentation. According to this analysis, virus isolated 20 days after inoculation consisted of only one component. Virus obtained from the inoculated leaf 5 days after inoculation contained a similar component. In addition, this virus preparation also contained a second component nearly double in length, as calculated from the rate of sedimentation. It was estimated that about one-third of the virus particles in this extract were present as the second component.

The larger component observed in these studies is not believed to be similar to the component previously described by WYCKOFF (**13**). He concluded that this component was not present in the plant but arose as a result of prolonged storage in contact with buffer salts used as solvent. In the current investigations the presence of the double-length component in only one of the two preparations would indicate that the long component observed in these studies was formed in the plant. If this component were due to mechanical treatment, it probably would have been present in both preparations, as both had been subjected to the same experimental manipulation and both had been stored less than 24 hours.

Discussion

Any attempt to explain the finding that tobacco-mosaic virus from newly formed lesions displays only a fraction of its potential biological activity is complicated by two divergent theories as to the nature of viruses. One theory holds that viruses are living organisms, though extremely small; the other that they are exceedingly large protein molecules.

If tobacco-mosaic virus is considered as a microorganism, it may be supposed that an increase of activity per unit of weight may result from the division of large particles into smaller ones with approaching maturity, thereby increasing the number of separate infectious particles. In the presence of abundant supplies of such materials as are needed for increase, division to form new individuals may not keep pace with enlargement and particles may attain a size and weight greater than that necessary for activity. Subsequent divisions may form two or more new and active, but smaller, units, accounting for the findings in the experiments with high-nitrogen plants. In the presence of a limiting factor, such as insufficient nitrogen, division of these large individuals may be retarded or even inhibited, giving rise to virus preparations of low activity, as found in the nitrogen-deficient plants.

If, on the other hand, the virus is a protein molecule, its formation may result from a series of reactions. The synthesis of a macromolecule may take place very rapidly but the macromolecule thus formed may not be the completed virus. Some slight change in structure may be necessary to activate this hypothetical precursor of the virus, and this reaction may take place more slowly. In such a process, it should be possible to isolate high-molecular weight particles very soon after inoculation, but not all of these would be infectious. The proportion of active particles would therefore increase with time. Virus preparations might consist, theoretically, wholly of inactive molecules at first, later of a mixture of inactive and active forms, and finally of active molecules alone.

It is obvious that additional explanations may be formulated under either

hypothesis regarding the nature of viruses. Until further evidence is obtained, however, multiplication of theories seems superfluous.

It is not necessary that the fundamental problem of virus nature be solved in order to confirm or refute such possibilities as have already been considered. Should smaller size of the active units with increasing maturity of the virus lesions be an adequate explanation of the activation demonstrated in these studies, a possibility strengthened by preliminary data from sedimentation constant determinations, particle-size studies using micrographs and other physical chemical techniques might serve as confirmation. Should, however, the activation of an inactive precursor be responsible for the phenomenon seen in aging infected leaves, chemical methods might need to be brought to bear on the problem.

Summary

Experiments have been carried out to study virus multiplication in the inoculated leaf and the effect of nitrogen starvation on virus thus formed. Turkish tobacco plants grown in nutrient sand cultures were inoculated with tobacco-mosaic virus on one of the lower mature leaves of each when the plants were about 6 inches tall. At intervals, virus was isolated by means of an ultracentrifuge and assayed for biological activity.

Virus in the inoculated leaf continued to increase both in amount and in specific biological activity (activity per unit of weight) for as long as 20 days after inoculation; withdrawal of the nitrogen supply inhibited the increase in specific activity earlier than it did the increase in virus protein. Confirmation of the finding that virus in young lesions displays only a fraction of its potential biological activity was obtained by comparing virus isolated from the inoculated leaf with virus isolated from the more recently invaded top of the plant.

Calculations from the rates of sedimentation in the analytical ultracentrifuge indicated that virus preparations from young lesions may contain some particles considerably larger than those characteristic of virus preparations from older lesions.

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

LITERATURE CITED

1. HOLMES, F. O. Local lesions in tobacco mosaic. Bot. Gaz. **87**: 39–55. 1929.
2. ————————. Local and systemic increase of tobacco mosaic virus. Amer. Jour. Bot. **17**: 789–805. 1930.
3. KUNKEL, L. O. Movement of tobacco-mosaic virus in tomato plants. Phytopath. **29**: 684–700. 1939.

4. NIGHTINGALE, G. T. The nitrogen nutrition of green plants. Bot. Rev. **3**: 85–174. 1937.
5. SAMUEL, G. The movement of tobacco mosaic virus within the plant. Ann. Appl. Biol. **21**: 90–111. 1934.
6. SESSIONS, A. C., and SHIVE, J. W. A method for the determination of inorganic nitrogen in plant extracts. Plant Physiol. **3**: 499–511. 1928.
7. SPENCER, E. L. The effect of host nutrition on concentration of tobacco-mosaic virus. Plant Physiol. **14**: 769–782. 1939.
8. ————————. Inhibition of increase and activity of tobacco-mosaic virus under nitrogen-deficient conditions. Plant Physiol. **16**: 227–239. 1941.
9. ————————. Correlation of activity per unit weight of tobacco-mosaic virus with age of lesion. Science n.s. **94**: 96–97. 1941.
10. ————————. The influence of nitrogen supply on rate of multiplication of tobacco-mosaic virus. Plant Physiol. **16**: 663–675. 1941.
11. ————————, and PRICE, W. C. Accuracy of the local lesion method for measuring virus concentration. I. Tobacco-mosaic virus. Amer. Jour. Bot. (In press.)
12. WATSON, RUTH, and PETRIE, A. H. K. Physiological ontogeny in the tobacco plant. 4. The drift in nitrogen content of the parts in relation to phosphorus supply and topping, with an analysis of the determination of ontogenetic changes. Australian Jour. Exp. Biol. and Med. Sci. **18**: 313–340. 1940.
13. WYCKOFF, R. W. G. Molecular sedimentation constants of tobacco mosaic virus proteins extracted from plants at intervals after inoculation. Jour. Biol. Chem. **121**: 219–224. 1937.

LIMITATIONS OF AUTO-IRRIGATORS FOR CONTROLLING SOIL MOISTURE UNDER GROWING PLANTS[1]

L. A. Richards and W. E. Loomis

(WITH FIVE FIGURES)

An accurate, convenient method for controlling soil moisture under growing plants would be of obvious advantage to the experimenter attempting to grow plants under uniform conditions, as well as to the physiologist who may wish to study the effect of varying soil moisture on plant development. Since the time of the original suggestion by Livingston in 1908 (**3**), various forms of auto-irrigators have been proposed for this purpose. Porous clay cones were later developed by Livingston (**4**) to overcome difficulties ascribed to poor contact between the soil and the porous water-supplying surface of the early cylindrical cups. Livingston, Hemmi and Wilson (**5**) used tensions up to 40 cm. of mercury and state, "The moisture conditions of the soil are now among the environmental conditions most susceptible of control and satisfactory measurement." Tensions of 60 to 65 cm. of mercury, equivalent to 8 or 9 meters of water, were considered to be maximum for satisfactory irrigator performance.

Hendrickson and Veihmeyer (**1**), with an unstated water supply tension, were unable to maintain uniform moisture distribution with irrigator cones, but found a layer of moist soil near the irrigator surface filled with absorbing roots. Under such conditions growth of plants might easily give indications of controlled low soil moisture which actually were only responses to a restricted rooting zone.

A substantial improvement in apparatus design was made with the development of double-walled irrigator pots by Korneff (**2**), Richards (**6**), Wilson (**14**) and Richards and Blood (**7**). Compared with porous irrigator cylinders or cones, these units maintain a larger fraction of the soil mass in proximity to and in good contact with the irrigator surface.

Wiggin (**11, 12**) has published results from a study of double-walled irrigators from the same source as those described by Wilson (**14**). He states that, "Soils can be held at an approximately constant percentage of soil moisture anywhere within a range from below the wilting point for plants to a condition of soil saturation." His data, however, hardly justify this conclusion for he reports, "The greatest difference found between the water content of the soil in the outside layer and the center of the pot was 4.2 per cent.; the greatest difference between the top and bottom layers of soil, 5.1 per cent. The variation in the water content of the soil . . . is therefore

[1] Journal Paper no. J–894, Iowa Agricultural Experiment Station, Ames, Iowa. Projects 487 and 678.

very small." A variation of 4 or 5 per cent. in as many inches, with the low percentage at or below the wilting point, would ordinarily not be considered small, and in a sandy loam soil, might represent the difference between the permanent wilting point and optimum soil moisture. Neither LIVINGSTON, HEMMI and WILSON, nor WIGGIN presents data showing that uniform pot weights were maintained.

This paper presents representative results from a series of experiments designed to test critically the usefulness and performance of improved double-walled irrigator pots under a variety of soil moisture and plant growth conditions. The results indicate definite limitations which are inherent in the moisture absorbing and transmitting properties of soil and which are not likely to be overcome by further improvements in irrigator design.

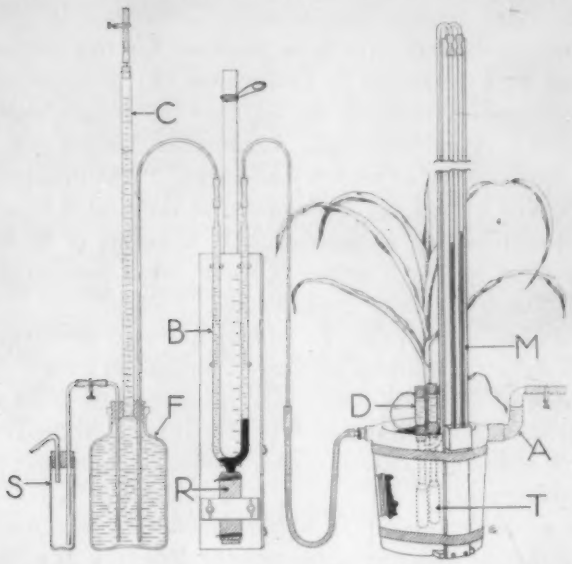


FIG. 1. Apparatus assembled for testing the performance of six-inch auto-irrigator pots. See text for description.

Apparatus

The double-walled irrigator pots[2] used were of single piece construction having a glazed outer wall ¼-inch thick, a porous inner wall ¼-inch thick, and an inter-wall space of ¼-inch for the supply water. Two sizes of pots were available: the soil cavities being 6 inches in diameter at the top, 5 inches in diameter at the bottom, and 6¾ inches deep in the smaller unit; and 10 inches in diameter at the top, 6 inches in diameter at the bottom, and 14

[2] These pots were designed by L. A. RICHARDS and manufactured by General Ceramics Company, New York, New York.

inches deep in the larger unit. The apparatus assembly used for the small-pot experiments is shown in figure 1. Any air entering the irrigator system accumulates in and may be removed from the glass air trap A. The Mariotte flask F serves as a constant pressure source of water which moves past the mercury in the barostat B to the water cavity in the pot. The mercury reservoir R provides a convenient adjustment for the supply water tension. The water uptake by the soil in the pot is measured in the burette C, and the safety flask S prevents loss of water from back flow which occasionally takes place because of thermal expansion.

Tensiometer cups T were installed in the pots, one at 2 cm. from the edge and the other in the center, to give information on soil moisture tension within the soil mass. In use the barostat was secured to the Mariotte flask with rubber bands and the flexible rubber tube connection made it possible to transfer the plant container to a balance for weighing. In the experiments reported in this paper a mixture of paraffin and petroleum jelly was used to prevent evaporation from the soil surface so that water losses were limited to transpiration.

Experiments with six-inch pots

Twelve six-inch pots were filled with Clarion loam, planted to maize, and thinned to three plants per pot. This soil had a wilting point of 7.0 per cent. and a field percentage of 13.1 per cent. Ammonium phosphate was mixed with the soil and the plants grew vigorously with a water tension of about one centimeter of mercury at the bottom of the pot.

On May 11, when the plants were 80 to 90 cm. from the soil surface to the tip of the tallest leaf, the barostats for six pots were set at 2.8 cm. of mercury and the remaining six pots were set at 4.2 cm. of mercury. Both sets of pots lost weight rapidly and in three days the pots with the 4.2 cm. barostat setting had lost 357 gm., equivalent to a change of 12 per cent. in the soil moisture, assuming uniform distribution within the pots, and the plants were wilting badly during the day. On May 17 the barostats were emptied on the 4.2 cm. pots and the water tension adjusted to zero at the bottom of the auto-irrigators. Only one of the six pots gained weight and the plants in the others continued to wilt. On May 22 the soil masses were removed from the irrigators and sampled for soil moisture at three locations in the pot: (a) at a point just above the center; (b) the outside centimeter of soil from the top half of the pot; and (c) the bottom centimeter of soil. The outside layer of roots was removed before taking samples (b) and (c), and large roots were removed from all samples. The data are shown in table I.

The three maize plants, about 85 cm. tall with a green weight of 30 gm. each, obviously utilized more water than could be supplied by the irrigator pot and uniform soil moisture conditions were not maintained. It is ap-

parent from the data that only one of the first six pots had wet up in the five days following complete removal of the supply water tension. This resistance of dry soil to wetting, aided in this instance by root mats and rapid transpiration, is a principal cause of failure in auto-irrigator systems. The soil moisture contents found on sampling were far below the equilibrium values of 20 to 22 per cent. that would have been maintained in the absence of plants, thus indicating definitely that the soil moisture was not being controlled by the irrigator, even with a barostat setting as low as 2.8 cm. of Hg. The literature on auto-irrigators contains no suggestion that such a barostat

TABLE I

PERCENTAGES OF "AVAILABLE"* SOIL MOISTURE UNDER MAIZE GROWING IN SIX-INCH AUTO-IRRIGATOR POTS

Pot number	Barostat tension in centimeters Hg		Percentage of available moisture in the soil on May 22		
	May 13–17	May 17–22	Center of pot	Outside top	Outside bottom
	cm.	*cm.*	%	%	%
1	4.2	0	1.8	2.3	8.9
2	4.2	0	21.2	23.2	20.9
3	4.2	0	1.7	2.1	10.5
4	4.2	0	3.5	5.6	15.3
5	4.2	0	2.7	2.5	8.7
6	4.2	0	5.5	7.0	13.7
7	2.8	2.8	7.2	6.5	18.5
8	2.8	2.8	1.9	1.9	7.8
9	2.8	2.8	2.1	2.0	2.5
10	2.8	2.8	2.9	2.0	2.0
11	2.8	2.8	10.8	16.3	19.5
12	2.8	2.8	3.8	7.8	12.1

* Soil moisture percentage minus the wilting percentage (7.0 per cent.).

setting is excessive for successful operation under the conditions of this experiment.

In spite of the nearly complete failure of the moisture supply in the center of the soil mass, the corn plants were making a satisfactory growth on moisture absorbed from the outer layers of soil or directly from the irrigator surface, and to the casual observer the experiment might have been considered normal. The pot-weight losses, tensiometer readings, and afternoon wilting of the plants, as well as the soil moisture percentages, showed clearly, however, that the soil moisture was out of control.

A number of auto-irrigator experiments with a range of soil and plant conditions have indicated that for any given rate of moisture absorption by roots, there is a definite upper limit for the supply water tension that will permit auto-irrigators to control even approximately the soil moisture per-

centage. The higher the rate of water extraction by roots, *i.e.*, rate of transpiration, the lower the supply water tension must be. This reciprocal relation of course was anticipated, but the authors were surprised to find how low the tension must be kept in the case of rapidly transpiring crop plants.

The curves in figure 2 give results from an experiment conducted in the greenhouse in December with one maize plant per six-inch pot. Procedures were as before with the same Clarion loam and barostat assembly. A series of experiments with these same plants in November resulted in irrigator failure, and all pots were flooded under pressure on November 27 to insure rapid and complete wetting of the soil. Barostats were attached

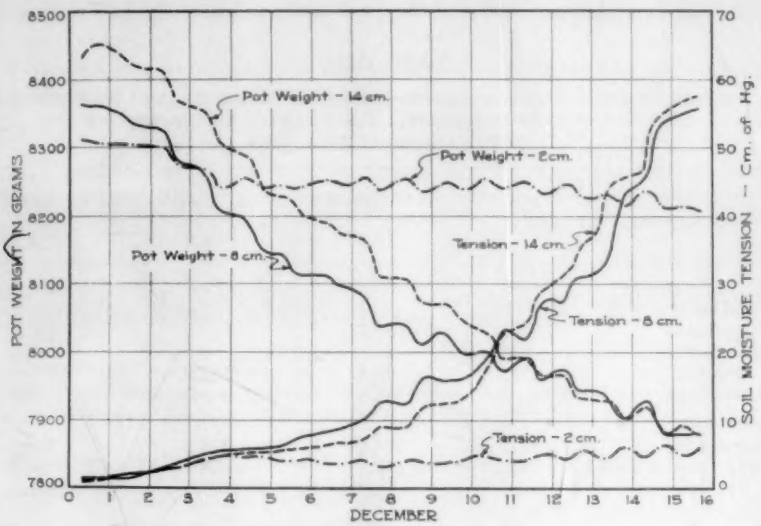


FIG. 2. Record of gross pot weight and soil moisture tension for three pots, each containing one maize plant 90 to 100 cm. tall. The barostat settings were 2, 8, and 14 cm. of mercury.

and adjusted on November 30 with four pots at each of three settings of 2 cm., 8 cm., and 14 cm. of mercury.

The curves of figure 2 show reasonably good control of pot weights with a 2.0 cm. barostat setting, with only one 30-gm. plant per pot and midwinter transpiration rates. Average weight dropped in 3 days from about 8300 to 8250 gm. and then fluctuated, losing by day and regaining by night, until December 12 when a gradual loss set in. The tensiometer curve is for the *outside* tensiometer which was placed 1.5 to 2.0 cm. from the irrigator wall. With a water supply tension of 2.0 cm. of mercury the soil moisture tension 2 cm. away varied between 4 and 6 cm. of mercury, which might be regarded as reasonably good control. At barostat settings of 8.0 and 14.0 cm. of mercury, however, the moisture content of the soil was definitely not maintained

or controlled. There was a continuous decrease in pot weight and a continuous increase in soil moisture tension.

Moisture movement data for the experiment are summarized in table II. They emphasize the limitations of auto-irrigators when considerable quantities of water must be transferred. The first period, Nov. 30 to Dec. 3, may be ignored as representing adjustments to changed barostat settings. During the second period, Dec. 4 to 9, the 2.0 barostat setting may be considered to have operated successfully with a soil moisture tension drop of about 1 cm. Hg per centimeter indicated by the outer tensiometer reading of 4.1 cm. Hg on Dec. 9. The 8.0-cm. series was going out of control with a tension difference of 4 cm. Hg per cm. and the 14.0 series apparently had not reached

TABLE II

TRANSPIRATION LOSSES AND AUTO-IRRIGATOR PERFORMANCE FOR SIX-INCH POTS CONTAINING ONE 90–100 CM. MAIZE PLANT. DATA ARE GRAMS PER POT PER DAY—AVERAGE OF FOUR POTS

PERIOD AND MEASUREMENT	BAROSTAT SETTING IN CENTIMETERS OF MERCURY		
	2.0	8.0	14.0
	gm.	*gm.*	*gm.*
Nov. 30–Dec. 3			
Transpiration	95.7	147.2	151.2
Uptake from reservoir	27.3	0.0	0.5
Net loss per pot	68.4	147.2	150.7
Soil moisture tension—Dec. 3	4.8	5.9	4.7
December 4–9			
Transpiration	273.2	236.3	272.3
Uptake from reservoir	259.2	55.3	30.6
Net loss per pot	14.0	181.0	241.7
Soil moisture tension—Dec. 9	4.1	15.6	12.7
December 10–15			
Transpiration	329.2	365.1	360.1
Uptake from reservoir	289.7	221.8	170.2
Net loss per pot	39.5	143.3	189.9
Soil moisture tension—Dec. 15	6.0	55.3	57.2

equilibrium although moisture was being absorbed from the irrigator while the soil moisture tension 2 cm. away was below the barostat reading. This situation was observed on several occasions and is ascribed to rapid absorption by roots in contact with the irrigator surface and to slow equalization of tensions within the soil mass. By December 15 the 2.0 cm. series was beginning to show failure, and the pots with the higher barostat settings were obviously completely out of control with soil moisture tension gradients of more than 20 cm. Hg per centimeter of soil.

In individual pots, tensiometer readings in excees of 60 cm. of mercury were obtained 2 cm. away from a water supply under a tension of only 8 cm. of mercury. Although transpiration losses were only 40 to 60 gm. per pot

per day and contact between the soil and irrigator was as good as can be obtained, the tensiometer and pot-weight readings and the soil moisture data of table III show that the soil moisture in all pots with as much as 8-cm. barostat setting was either out of control or rapidly failing.

The third column of the table labeled "static equilibrium moisture" shows the moisture content that was obtained when the soil without plants was saturated and allowed to come to equilibrium with supply water at the tensions shown in column two. Only pot no. 1 maintained this equilibrium percentage, while pots 5 to 12 showed significant moisture losses in even the bottom centimeter of soil, with other losses running above 70 per cent. of

TABLE III

AVAILABLE SOIL MOISTURE PERCENTAGES IN THE SIX-INCH POTS ON DECEMBER 15

POT NUMBER	BAROSTAT TENSION IN CM. HG	STATIC EQUILIBRIUM MOISTURE†	PERCENTAGE OF AVAILABLE SOIL MOISTURE*		
			CENTER OF POT	OUTSIDE TOP	OUTSIDE BOTTOM
	cm.	%	%	%	%
1	2.0	22.0	22.7	22.2	23.6
4	2.0	22.0	17.0	15.6	24.2
7	8.0	18.7	8.4	9.5	15.2
8	8.0	18.7	6.0	7.1	15.1
10	14.0	16.8	8.1	8.6	12.3
12	14.0	16.8	5.7	4.8	9.3

* Total moisture percentage less the wilting percentage (7.0 per cent.).
† As determined in unplanted auto-irrigator assemblies.

the theoretical values at the end of a two-week period, and with no important tendency for losses to be regained.

Experiments with ten-inch pots

Experiments with ten-inch double-walled pots likewise have shown that auto-irrigators will function successfully only when both the supply water tension and the rate of water extraction by roots is low. Detailed information on the performance of individual ten-inch irrigator pots has been obtained by means of an apparatus assembly (**8**) whereby the pot weight and the water input to the pot were automatically and continuously recorded. Continuous records were also kept of air temperature, humidity and atmometer evaporation. The curves in figure 3 show typical results for the ten-inch pots. The gross pot weight and the rate of water input to the pot are shown for three soybean plants growing in Clarion loam soil. The tension in the water at the bottom of the pot was maintained at 1.5 cm. of mercury throughout the test. It was found in this particular experiment that, except for small diurnal fluctuations, the irrigator was able to maintain constant

pot weight until the transpiration rate for the three soybean plants reached about 360 ml. per day.

The seventh day shown in the figure was hot and dry and the transpiration loss from the plants exceeded the amount of moisture the soil was able to absorb from the irrigator during the following night. From this time on there was a downward trend in the pot weight, no substantial pot weight recovery being attained even after a succession of low transpiration days such as the fifteenth and sixteenth in the figure.

At the end of the period shown in figure 3 the experiment was terminated. The soybean plants growing in the pot were 82 cm. high and were making vigorous growth. The roots were well nodulated and evenly distributed throughout the pot. There was some concentration of roots at the bottom

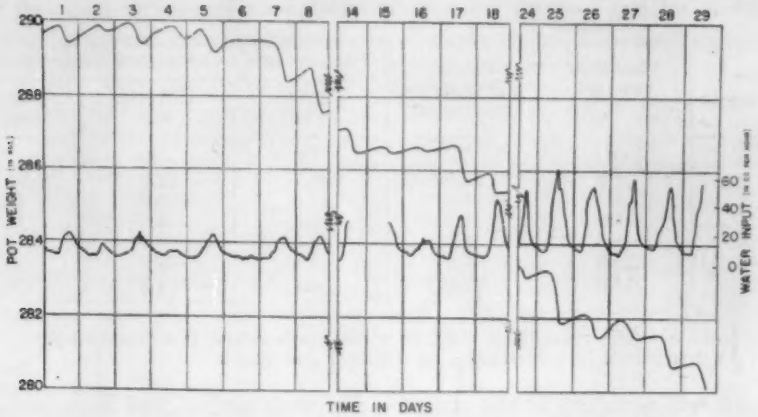


FIG. 3. Rate of water input and gross weight for 10-inch irrigator pot containing three soybean plants.

of the pot and around the side, but not sufficient to prevent excellent contact between the soil and the porous wall of the irrigator. In spite of the large moisture supplying surface and the low tension of the supply water, the steadily declining gross pot weight indicates that the moisture content of the soil was not being maintained by the irrigator. The moisture content at the center of the pot at take down was 12.9 per cent., whereas at the top-outside and bottom-outside edges of the soil mass the moisture percentages were 15.7 and 18.1 respectively.

Experiments with oats, wheat, soybeans, and maize in double walled irrigator pots using various soils were consistent in showing that when the moisture tension in the surrounding soil or the irrigator wall exceeded two or three centimeters of mercury (27 to 40 cm. of water) moisture transfer did not take place rapidly enough to prevent the soil around the roots from becoming progressively drier. This change was particularly marked when

the transpiration rate was high. Better control was obtained in loam soils of medium texture than in coarser or finer soils, but the best results obtained in a dozen experiments with crop plants were decidedly disappointing.

Discussion

An auto-irrigator pot makes it possible to surround a test mass of soil with what is equivalent to a mass of soil at constant moisture content and, in addition, provides means for measuring the average content of moisture and the capillary transfer of water to the test mass in which roots are growing. The results of these experiments, which appear also to apply directly

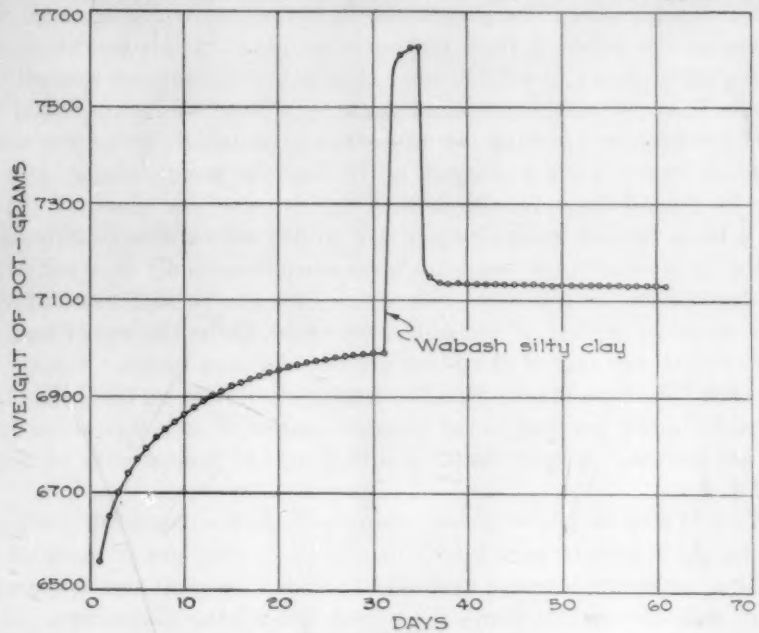


FIG. 4. Changes in pot weight show directly the moisture transfer to and from a mass of soil contained in a six-inch irrigator pot. The tensions of the supply water for the three sections of the curve were 22, 0, and 22 cm. of mercury, respectively. At the start the soil was air dry with a moisture content of 4.3 per cent.

to field conditions, indicate that if the moisture tension in the surrounding soil is more than a few centimeters of mercury, moisture transfer does not take place rapidly enough to prevent the progressive depletion of moisture from soil near the root systems of developing crop plants.

It is this slowness of transfer of water through unsaturated soil coupled with a certain "resistance" of soils to rewetting that limits the range of usefulness of double-walled irrigator pots.

The authors have no suggestions to offer for the improvement of the auto-irrigator units used in these experiments. The mechanical and physical

properties and shape of the porous water-supplying surface seemed fairly ideal. No trouble was experienced with leaks or breakage. Since the soil moisture tension and moisture content ranges over which auto-irrigators can be successfully used appear to be limited primarily by the moisture absorbing and transmitting properties of the soil, it seems appropriate to review briefly some of these properties.

The rate at which an air dry soil absorbed moisture when placed in a six-inch irrigator pot and protected from evaporation is shown in figure 4. The irrigator initially was supplied with moisture at a tension of 22 cm. of mercury, and even after 30 days the soil moisture percentage had not fully reached equilibrium. The abrupt rise in the weight curve occurred when all tension was removed from the water supply. In this condition rapid wetting takes place and equilibrium soil moisture content was reached in 48 hours or less. When the tension of the supply water was again raised to 22 cm. of mercury, by lowering the water supply container, water was rapidly extracted from the soil and again no further pot weight change occurred after the second day. Numerous such curves have been obtained (**9**) and give a basis for the generalization that under comparable conditions soil wetting by capillary processes takes place much less rapidly than soil drying. The decrease in the readiness with which soils absorb and transmit water as the moisture content of the soil is decreased, limits the use of auto-irrigators to the wet end of the plant growth moisture range. WILSON and RICHARDS (**13**) have summarized the experimental data on this subject. A decrease of a few per cent. in the moisture content of soils may cause a hundredfold decrease in the moisture conductivity, or permeability to unsaturated flow.

While it may be said that low conductivity makes impossible the proper functioning of auto-irrigators with drier soils, it must not be assumed that moisture movement becomes insignificant as far as plant use is concerned at any moisture content above the permanent wilting percentage. It has been found for instance with pressure-membrane apparatus (**10**) that layers of soil one centimeter thick can be dried from saturation to below the wilting percentage in 24 hours or less, thus indicating that while moisture movement at or near the wilting point may be slow it is not absent.

We have emphasized conditions under which auto-irrigators have failed to operate successfully because the limitations of auto-irrigators do not appear to have been adequately presented in the literature. Auto-irrigators may be expected, however, to give satisfactory moisture control whenever the rate at which moisture can be transported from the irrigator wall through the soil is greater at all times than the maximum rate at which moisture is extracted from the soil. In some kinds of work where constant moisture conditions are not required the desired moisture control may be

obtained by alternately raising and lowering the tension of the supply water of the irrigator. The water-supply arrangement shown in figure 1 is more elaborate than is ordinarily required and can be replaced by a simple water connection to a reservoir at a lower level (fig. 5). A pointer, equivalent to a hook gage, can be used for accurate refilling of the reservoir when water use measurements are desired. An especially valuable feature of this assembly

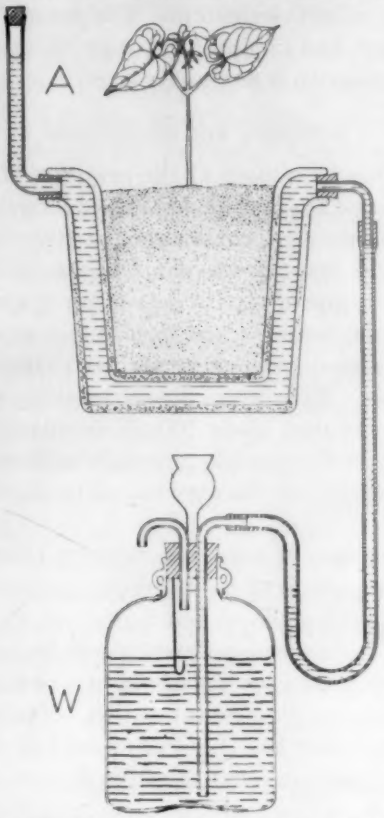


FIG. 5. A convenient assembly for growing plants in auto-irrigators. Water tension is varied by raising or lowering the reservoir W.

is its rapid drainage to equilibrium (compare fig. 4), so that surface watering may be used at will without appreciably affecting the experimental conditions.

It is not possible at present to enumerate the conditions under which auto-irrigators will work satisfactorily because in addition to the irrigator, the soil, the plant, and the entire physical environment are involved. The following, however, appear to be types of experimental work for which auto-irrigators may be successfully used for varying or controlling soil moisture:

seed germination; early seedling growth; rooting of cuttings or work with plants that transpire slowly and do not rapidly develop root mats and become potbound; and the study of the development and control of soil-borne insects and pathogens when cultured in the absence of rapidly transpiring plants. Also the study of soil bacterial processes, nitrogen transformations, organic matter decomposition and carbon dioxide evolution may be greatly facilitated by the use of auto-irrigators. The porous wall of the irrigator acts as a bacterial filter, and this property may be of use in certain experiments where sterile irrigation is necessary.

Summary and conclusions

A detailed study has been made of the precision of moisture control attainable with six-inch and ten-inch double-walled irrigator pots. Maize, soybeans, wheat, and oats were grown in various soils. Records were kept on gross pot weight, water input to the pot, and tension in the soil water, as well as air temperature, humidity, and atmometer evaporation rate. It was found with low barostat tensions that good moisture control was attained while the plants were small, and there was no change in the average pot weight from day to day. With larger plants, however, when the daily transpiration loss was greater than about 100 ml. per day (350 ml. per day for the 10-inch pots), it was not possible to maintain the soil moisture content, even with the supply water for the reservoir at tensions as low as 2 to 4 cm. of mercury.

The range of usefulness of auto-irrigators for controlling soil moisture in plant experiments appears to be definitely overrated in the literature. Satisfactory growth can be obtained and the rate at which the plants absorb water varied, but soil moisture under crop plants having high transpiration rates and rapidly developing root systems cannot be maintained at any reasonably constant value except near saturation. The moisture tensions of 40 to 60 cm. of mercury that have been mentioned in the literature are far beyond the successful moisture control range of auto-irrigators containing rapidly transpiring plants.

The limitation in the range of successful operation of irrigators may be ascribed primarily to the slow rate at which drying soils absorb and transmit water, particularly when the soil moisture is under appreciable tension. The hysteresis effect in the relation between the soil moisture tension and the moisture content accounts in part for the fact that it is difficult for an auto-irrigator to rewet soil from which the moisture has been depleted by roots. Also, a decrease of a few per cent. in the moisture content may cause a hundredfold decrease in the moisture conductivity or permeability of soils for unsaturated flow, and local decreases are probably characteristic of the soil immediately surrounding all roots that are absorbing moisture rapidly.

The design and construction of the irrigator pots used are fairly ideal.

No trouble was experienced with leaks or cracks. These units appear to have definite usefulness in soil moisture control problems that do not require the rapid movement of moisture through soil.

IOWA AGRICULTURAL EXPERIMENT STATION
AMES, IOWA

LITERATURE CITED

1. HENDRICKSON, A. H., and VEIHMEYER, F. J. Influence of dry soil on root extension. Plant Physiol. **6**: 567–576. 1931.
2. KORNEFF, B. J. La capacité d'absorption du sol, appareils pour la mesurer—arrosage automatique. Ann. Sci. Agron. **43**: 353–385. 1926.
3. LIVINGSTON, B. E. A method for controlling plant moisture. Plant World **11**: 39–40. 1908.
4. ————————. Porous clay cones for the auto-irrigation of potted plants. Plant World **21**: 202–208. 1918.
5. ————————, HEMMI, T., and WILSON, J. D. Growth of young wheat plants in auto-irrigated soils as related to the water-supplying power of the soil and to the adjustment of the auto-irrigator. Plant Physiol. **1**: 387–395. 1926.
6. RICHARDS, L. A. The usefulness of capillary potential to soil-moisture and plant investigators. Jour. Agr. Res. **37**: 719–742. 1928.
7. ————————, and BLOOD, H. L. Some improvements in auto-irrigator apparatus. Jour. Agr. Res. **49**: 115–121. 1934.
8. ————————, and RUSSELL, M. B. Apparatus for studying water-relations in potted plants. Trans. Amer. Geophysical Union, 18th Annual Meeting, 588–592. 1937.
9. ————————. Uptake and retention of water by soil as determined by distance to a water table. Jour. Amer. Soc. Agron. **33**: 778–786. 1941.
10. ————————. A pressure-membrane apparatus for extracting soil solutions. Soil Science **51**: 377–386. 1941
11. WIGGIN, W. W. Effect of variations in the soil water content on the growth of certain species of plants. Thesis, Ohio State University, Columbus, Ohio. 1935.
12. ————————. Effects of variation in the soil water content on the growth of certain species of plants. Ohio State Agr. Exp. Sta. Bi-monthly Bull. **22**: 136–141. 1937.
13. WILSON, B. D., and RICHARDS, S. J. Capillary conductivity of peat soils at different capillary tensions. Jour. Amer. Soc. Agron. **30**: 583–588. 1938.
14. WILSON, J. D. A double-walled pot for the auto-irrigation of plants. Torrey Bot. Club Bull. **56**: 139–153. 1929.

GROWTH, AUXIN, AND TROPISMS IN DECAPITATED AVENA COLEOPTILES

F. W. WENT

(WITH THREE FIGURES)

With all the work on auxin appearing in the last years it is remarkable that not more investigations are concerned with the actual rôle of the auxins inside the plant. Barring some early opposition, the original statement "Without auxin no growth" (**12**) has stood unchallenged for many years. Probably this was so because all test methods were based on the principle of proportionality between auxin applied and its resultant effect. When BONNER and THIMANN (**2**) established that there was also direct proportionality between auxin used up and growth, the paramount importance of auxin in the growth process seemed firmly established. In later years, however, doubts have been expressed about the necessity of auxin for the growth process in general. AVERY and LARUE (**1**) concluded "that growth hormone is not a necessity for elongation of the coleoptile." The confusion was due to the fact that auxin is present inside the plant in different states. This was pointed out especially by WENT and THIMANN (**15**), and WENT (**13**), who stressed the point that it is essential to distinguish between auxin which can be collected by diffusion (diffusible or free auxin) and the auxin which is obtained by extraction. It is evident that only that part of the auxin which combines in some way inside the cells can be responsible for growth, whereas correlation phenomena such as tropisms can be brought about only by a correlation carrier which moves freely inside the tissues.

Since no attempt has been made to correlate for one experimental object the relations between diffusible auxin, extractable auxin, growth rates, and tropisms the following paper describes experiments in which this was done for the Avena coleoptile. This is an especially favorable object for such studies since its growth rate and tropistic behavior can be radically changed by simple decapitation. The relation between auxin production and growth and tropisms was worked out by DOLK (**3**). After decapitation the growth rate of the Avena coleoptile falls off until two and one-half hours later when the growth rate suddenly rises to a value somewhat below its original rate. Geotropic and phototropic sensitivity show the same general dependence upon decapitation. Whereas tropistic sensitivity disappears immediately after cutting off the tip, it suddenly reappears two and one-half hours later. This behavior can be correlated with the auxin production in a coleoptile. Decapitation removes the production center of auxin, but two and one-half hours after removal of the tip, the cells immediately below the cut surface

regain the ability to produce auxin. This is called regeneration of the physiological tip. Each of these phenomena will be measured quantitatively in the following investigation.

Auxin content of decapitated coleoptiles

The extractable auxin from coleoptile zones 5 to 25 mm. below the original tip was determined by ether extraction. Thirty-six to 48 coleoptiles were cut off and placed in freshly prepared ether and left overnight. According to VAN OVERBEEK (11), under those conditions at least 75 per cent. of all free auxin present is extracted from coleoptiles. Extractions were made of five separate lots before, and one, two, three, and four hours after decapitation. The extracts were then taken up in 0.5 ml. of 1.5 per cent. agar and tested on 24 test plants. The results of two experiments are given in table I. They show that the auxin content of coleoptiles gradually decreases to 50 per cent. in the first two hours after decapitation. After 3

TABLE I

AUXIN CONTENT OF AVENA COLEOPTILES, INCLUDING THE REGION 5 TO 25 MM. FROM ORIGINAL TIP ONLY, WHEN EXTRACTED WITH ETHER AT VARIOUS TIMES AFTER DECAPITATION. EXTRACT TAKEN UP IN 0.5 ML. AGAR, AND CUT INTO AGAR BLOCKS OF 12 MM.[3]

	Experiment 1				Experiment 2				
	Number of coleoptiles extracted	Mean Avena test curvatures per 12 test plants	Curvature per 100 extracted coleoptiles	Auxin content; percentage of intact coleoptiles	Number of coleoptiles extracted	Avena test curvature	Curvature per 100 extracted coleoptiles	Auxin content; percentage of intact coleoptiles	Auxin content; mean of expts. 1 and 2
		deg.	*deg.*	%		*deg.*	*deg.*	%	
At the moment of decapitation	36	7.3, 7.9	21.0	100	36	6.7	18.6	100	100
1 hour after decapitation	48	6.9, 6.9	14.4	69	48	6.7	14.0	75	72
2 hours after decapitation	48	4.5, 5.0	9.9	47	48	5.0	10.4	56	51
3 hours after decapitation	48	8.1, 8.3	17.1	81	48	8.2	17.1	92	86
4 hours after decapitation	36	4.9, 6.4	15.7	75	36	6.9	19.2	103	89
25 γ/L indole-acetic acid	.....	7.2	.....	.....	.....	.....	.....	.....	.....
50 γ/L indole-acetic acid	.....	13.7	.....	.....	.....	18.7	.....	.....	.....
100 γ/L indole-acetic acid	.....	.....	.....	.....	.....	24.2	.....	.....	.....

hours there is a sudden increase to more than 80 per cent. of the original auxin content. How reproducible these results are is evidenced by the fact that BONNER and THIMANN (2) found that, irrespective of whether coleoptiles are in contact with the seeds or cut off, about 50 per cent. of the extractable auxin has disappeared 2 hours after decapitation. After 3 hours they found that the auxin content had risen again to 83 per cent. of normal. Their experiments were carried out 5 years earlier with the chloroform extraction method, but their Avena plants had been grown under essentially the same conditions of temperature, light, and humidity.

Growth rate after decapitation

Coleoptiles of the same age and size as used for the auxin extractions were measured with the horizontal microscope to determine their growth rate. The results of these measurements are presented in figure 1 and

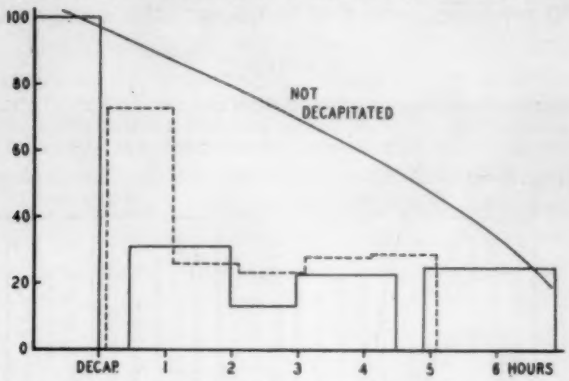


FIG. 1. Growth rates of decapitated Avena coleoptiles, expressed (on ordinate) in per cent. of the rate before decapitation. The upper curve represents growth rate of intact control plants. For actual values see table II. The dotted block diagram gives the results of another experiment.

table II; their behavior can be compared directly with the plants used for the auxin extractions in table I. After decapitation, growth decreases first slowly and then more rapidly until after 3 hours an increase occurs due to regeneration of the physiological tip. Reference to the data of DOLK (3) shows that the plants behaved normally. With the help of his data the block diagram of figure 1 has been smoothed to obtain the growth rate curve which is used for comparison with the auxin content, auxin production, etc. (fig. 3). It will be seen from table II that the growth rate of the non-decapitated control plants fell off considerably after six hours, due to the grand period. Therefore, the growth rate of the decapitated plants was not expressed in percentage of the original rate but as the percentage of the growth rate of the non-decapitated controls. Without this correction the

shape of the curve is essentially the same but remains at a somewhat lower level.

The growth rate of coleoptiles depends not only on their auxin content but also upon their response to the available auxin. To obtain figures which would permit one to estimate the sensitivity of coleoptile cells to auxin after decapitation, the same concentration of auxin (agar blocks containing 50γ

TABLE II

GROWTH RATE IN MM./HOUR OF NORMAL AND DECAPITATED AVENA COLEOPTILES* (SAME GROUP AS IN EXPERIMENT 1, TABLE I)

	GROWTH RATE				
	BEFORE DECAPITATION	AFTER DECAPITATION			
	1 HOUR	0–2 HOURS	2–3 HOURS	3–4½ HOURS	5–7 HOURS
	mm./hr.	*mm./hr.*	*mm./hr.*	*mm./hr.*	*mm./hr.*
Not decapitated, intact plants	1.41	1.17	1.05	0.79	0.46
Decapitated plants	1.46	0.45	0.19	0.34	0.36
AUXIN APPLIED†	PERCENTAGE INCREASE IN GROWTH RATE DUE TO AUXIN APPLICATION‡				
	%	%	%	%	%
2 hours after decapitation	.....	.....	37	100	53
3 hours after decapitation	.....	.....	.....	38	30
5 hours after decapitation	.....	.....	.....	.....	25

* Only the zones 5 mm. below the tip are measured.

† Various times after decapitation an agar block containing 50γ/liter indoleacetic acid was applied to the cut surface and renewed every two hours.

‡ Growth increase due to auxin application is indicated as percentage of growth rate of decapitated plants in the same period.

indoleacetic acid per liter, renewed every 2 hours) was applied to the entire cut surface of coleoptiles at various periods after decapitation. The growth rate of decapitated coleoptiles treated in this way was expressed as the per cent. of the growth rate of coleoptiles which did not receive this additional auxin. From table II it appears that the greatest response is obtained when auxin is applied two hours after decapitation; after 3 and 5 hours, however, the response was reduced. This phenomenon has been investigated before and has been called aging (**4**). In the regular Avena test the sensitivity of auxin greatly increases during the first three hours after decapitation, but this phenomenon is due to the decreasing auxin content of the cells (**7**).

Diffusible auxin

Avena coleoptiles were cut into a number of cylinders and the auxin diffusing out of different levels of the coleoptile was measured. This was done by cutting 24 coleoptiles into 2-mm. sections; these cylinders cut at the same level were placed together with their bases down on the standard 8 × 10.5-mm. agar plate. The latter was cut into 12 blocks and each block was applied to a standard Avena test plant. After the cylinders had stood for 2 hours on one agar plate they were transferred to another. In this way the amount of auxin diffusing out of the coleoptile cylinders in 2-hour periods was measured. This value corresponds with the auxin production. Some of the results are shown in table III. Coleoptile tips of 2 mm. length continued to produce auxin for the first 4 hours, but in the 2 hours immediately following decapitation the coleoptile cylinders, whether cut 2, 4, or 8 mm. below the tip, did not produce any measurable amount of auxin. This is in complete accord with earlier work (**12**). In the next 2-hour period these same coleoptile cylinders started to produce considerable amounts of auxin (15 to 25 per cent. of the production by tips). Comparison with other data (**11**) shows that the same holds true for corn coleoptiles. Only when the cylinders were more than 8 mm. removed from the tip did the regeneration of auxin production not occur within the first 4 hours. It was conceivable that auxin was produced by the coleoptile cylinders in the first 2 hours after cutting, but in such small amounts that the standard Avena test did not indicate them. Therefore, the same experiment was repeated using the deseeded Avena test (**9**). Table IV shows the results of a comparison of both tests, carried out on the same day and with the same material. The amounts

TABLE III

AUXIN PRODUCTION BY COLEOPTILE CYLINDERS. THE AUXIN DIFFUSING IN TWO HOURS TIME FROM 24 TWO-MM. LONG COLEOPTILE CYLINDERS, IS EXPRESSED IN DEGREES STANDARD AVENA TEST CURVATURE. THE CYLINDERS WERE EITHER CUT OFF IMMEDIATELY OR TWO HOURS AFTER DECAPITATION. FIGURES IN ITALICS INDICATE AUXIN REGENERATION

	Distance of cylinders from original tip	Hourly period after decapitation		
		0–2	2–4	4–6
	mm.	*deg.*	*deg.*	*deg.*
Tips of plants 2 mm. long	0	21.6	19.8	.....
Coleoptile cylinders from same plants	2–4	0.3	*4.6*	.....
Next cylinders	4–6	+0.3	*3.0*	.....
Cylinders from same plants	8–10	0.0	0.3	.....
Apical coleoptile cylinders cut off 2 hrs. after 2-mm. decapitation	2–4	.....	*2.8*	6.9
Apical coleoptile cylinders cut off 2 hrs. after 8-mm. decapitation	8–10	.....	0.6	*8.8*

of auxin are expressed in indoleacetic acid equivalents, which rules out the individual differences in sensitivity of both tests.

TABLE IV

AUXIN DETERMINATIONS, BOTH WITH THE STANDARD AND DESEEDED AVENA TEST, OF THE MATERIAL DIFFUSING OUT OF AVENA COLEOPTILES. AUXIN EXPRESSED AS INDOLEACETIC ACID EQUIVALENTS, WHICH OBVIATES DIFFERENCES IN SENSITIVITY BETWEEN THE TWO TESTS

	EXPERIMENT 1		EXPERIMENT 2		MEAN OF BOTH EXPERIMENTS	
	STANDARD	DESEEDED	STANDARD	DESEEDED	STANDARD	DESEEDED
Auxin produced by 36 coleoptile tips	90	72	51	69	70	70
Auxin produced by 36 coleoptile cylinders 2 to 4 mm. from tip, 0 to 2 hrs. after cutting	<11	22	<10	16	<10	19
Auxin produced by same coleoptile cylinders 2 to 4 hours after cutting	62	51	42	40	52	46

It is evident that whenever auxin is produced by coleoptile tips and coleoptile cylinders after regeneration, the standard and deseeded Avena tests give approximately the same results. Only the values for auxin production in coleoptile cylinders during the first 2 hours after decapitation differ. This indicates that for the first period the curvature in the deseeded test is

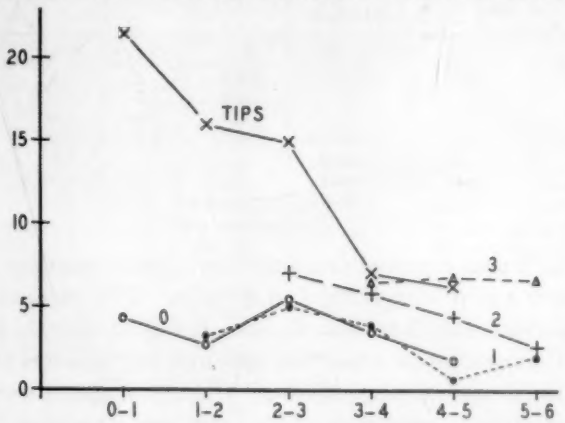


FIG. 2. Amount of auxin (ordinate: degrees curvature per 12 Avena test plants), diffusing out of 36 coleoptile cylinders in successive hourly periods (abscissa). Two millimeters of tip cut off, the next two-mm. sections were cut in curve 0 (circles) at the moment of decapitation, curve 1 (solid dots) one hour, curve 2 (plusses) two hours, and curve 3 (triangles) three hours after decapitation. For comparison the amount diffusing out of tips is plotted (crosses).

almost exclusively due to auxin precursor and not to free moving auxin, for the deseeded test responds not only to auxin but also to auxin precursor.

With this fact in mind we can now consider the data of table V and figure 2. Because of the greater sensitivity of the deseeded test the 24 cylinders were moved once every hour to a fresh agar plate, and thus the auxin production could be followed more closely. Since we have seen that the response of the deseeded plants in the first 2 hours after decapitation is due to auxin precursor we can conclude that the apical coleoptile cylinders give off more precursor, which is in excellent agreement with the experiments of VAN OVERBEEK (11) who found that the region nearest the tip contained most precursor. Regeneration of auxin production occurs after 2 to 3 hours

TABLE V

AUXIN PRODUCTION BY COLEOPTILE CYLINDERS. CURVATURES IN DEGREES PER 24 TWO-MM. SECTIONS PER AGAR PLATE PER HOUR DIFFUSION, TESTED ON DESEEDED AVENA PLANTS. THE FIRST FOUR GROUPS WERE TIPS AND SECTIONS ALL CUT FROM THE SAME PLANTS; THE NEXT THREE GROUPS WERE APICAL SECTIONS FROM THREE SEPARATE GROUPS OF 2 HOURS PREVIOUSLY DECAPITATED PLANTS. VALUES ITALICIZED CORRESPOND WITH REGENERATED AUXIN

	Length of decapitated tip	Hours elapsed between decapitation + cutting of sections	Distance from original tip	Hourly periods after decapitation				
				1	2	3	4	5
	mm.	*hr.*	*mm.*	*deg.*	*deg.*	*deg.*	*deg.*	*deg.*
Tips	..	0	0–2	24.8	.....	17.4	.....	6.0
Next 2 mm.	..	0	2–4	6.3	2.5	*3.9*	2.4	1.4
Next 2 mm.	..	0	4–6	4.9	2.0	*4.6*	.....	1.2
Next but 2 mm.	..	0	8–10	3.5	0.2	0.2	*3.2*	.....
Apical sections	2	2	2–4	.....	.....	*6.8*	6.8	4.6
Apical sections	4	2	4–6	.....	.....	2.6	*7.5*	3.8
Apical sections	8	2	8–10	.....	.....	1.6	1.1	*1.9*

in sections 2 to 6 mm. removed from the tip, whereas sections 8 to 10 mm. away regenerate auxin only after 3 to 4 hours. This compares very well with the data from TSI TUNG LI (6) who found at 20° C. (with growth measurements) regeneration after 143, 166, and 182 minutes for 2-, 4-, and 6-mm. decapitated plants, respectively. When the apical sections are removed 2 hours after decapitation, these times are somewhat longer which fact was confirmed in other experiments.

From these same experiments it also follows that the amount of auxin which is regenerated by the apex of decapitated plants is larger the longer the sections are left on the plants. This is particularly clear in figure 2, and has been confirmed in other experiments such as shown in table VI.

TABLE VI

AUXIN PRODUCTION (IN DEGREES PER 12 DESEEDED PLANTS) PER 24 APICAL COLEOPTILE SECTIONS, 2 TO 4 MM. BELOW THE ORIGINAL TIP, WHEN THEY ARE CUT OFF AND PLACED ON AGAR 0, 1, 2, OR 3 HOURS AFTER DECAPITATION. SECTIONS REPLACED ON NEW AGAR PLATES EVERY TWO HOURS

	TIME AFTER DECAPITATION	HOURLY PERIODS AFTER CUTTING APICAL SECTIONS					
		EXPERIMENT 1			EXPERIMENT 2		
		0–2	2–4	4–6	0–2	2–4	4–6
	hr.	*deg.*	*deg.*	*deg.*	*deg.*	*deg.*	*deg.*
Coleoptile tips	...	24.1	10.3	2.3	31.8	29.5	14.5
2-mm. apical section	0	1.4	6.1	0.5	7.7	6.3	3.0
" " "	1	4.0	3.8	.....	10.2	8.2	.....
" " "	2	6.0	10.8	.....	14.2	7.2	.....
" " "	3	8.4	.....	.....	12.7	.....	.....

This means that after decapitation auxin precursor continues to accumulate in these apical sections. The same conclusion was reached by VAN OVERBEEK (11) for corn coleoptiles.

Geotropism and phototropism of decapitated coleoptiles

DOLK (3) found that within the first hours after decapitation only very slight curvature resulted from geotropic stimulation. Moreover, these curvatures appeared mainly in the basal zones. Six hours after decapitation completely normal geotropic curvatures could be observed. A number of

TABLE VII

GEOTROPIC AND PHOTOTROPIC CURVATURES OF DECAPITATED AVENA COLEOPTILES, MEASURED 70 OR 120 MINUTES AFTER BEGINNING OF EXPOSURE. EACH FIGURE IS THE MEAN OF 10–12 PLANTS. THE FIRST FIGURES SHOWING REGENERATION HAVE BEEN ITALICIZED

EXPOSURE OF COLEOPTILES AFTER DECAPITION	CURVATURES IN DEGREES				
	GEOTROPIC CURVATURES 15 MINUTES HORIZONTAL EXPOSURE			PHOTOTROPIC CURVATURES 5 MM. DECAPITATED 10 MINUTES EXPOSURE	
	2 MM. DECAP.	5 MM. DECAP.	8 MM. DECAP.	60 METER-CANDLES	2 METER-CANDLES
	deg.	*deg.*	*deg.*	*deg.*	*deg.*
Not decapitated	10.2	9.1	9.1	7.4	28.9
2 minutes	6.8	.....	.....	.....	.....
10 "	5.1	3.0	.....	1.8 (base)	−0.4
30 "	1.6	3.0	.....	2.3 (base)	0.9
60 "	1.8	1.7	.....	.....	.....
80 "	.....	.....	.....	1.6	*3.6* (top)
90 "	*4.6*	1.2	1.5	.....	.....
120 "	7.3	*3.5*	0.4	*2.0* (top)	4.3 (top)
150 "	7.6	4.1	*3.8*	.....	.....
180 "	.....	.....	.....	3.3 (top)	3.0 (top)

experiments were carried out to determine the extent of geotropic curvature in coleoptiles of which 5 mm. had been removed. Only a few results are reproduced in table VII. It was found that immediately after decapitation a fair geotropic sensitivity still existed which rapidly disappeared within the next half hour. All these geotropic curvatures after decapitation appear low down in the base, but after 120 to 150 minutes ordinary geotropic sensitivity reappears resulting in curvatures near the apical cut surface. The shorter the decapitated tip was, the earlier the geotropic sensitivity reappeared.

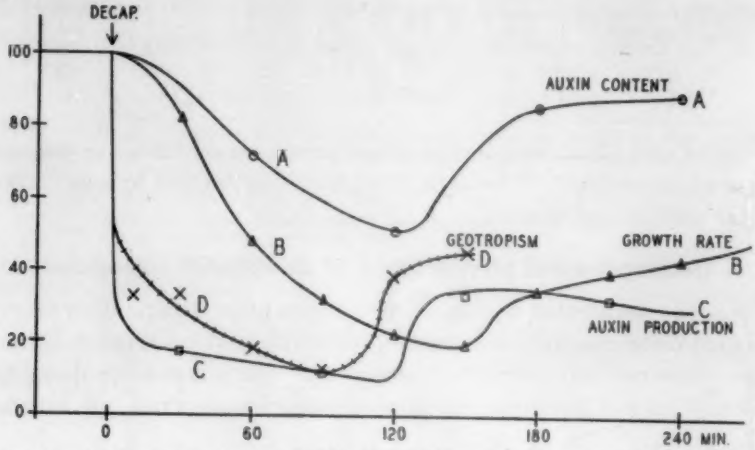


FIG. 3. Auxin content as determined by ether extraction curve A, growth rate B; curve C represents auxin production of coleoptile rings 5 mm. below tip as determined by diffusion into agar, and D geotropic response of Avena coleoptiles, in the 4 hours following decapitation of a 5-mm. long tip. All values expressed as percentage of values before decapitation (ordinate). For further explanation see text.

Almost the same behavior was observed for phototropic curvatures provided that only limited amounts of light were used and when a differentiation between the basal curvatures and the curvatures first appearing near the apex was made. Under such conditions it is easy to determine when the phototropic curvatures increase again as a result of regeneration of auxin by the apical cut surface. Because of the complexity of the phototropic response, however, no further data will be given since the details of the experiments would only confuse the main issue. It suffices to state that immediately after decapitation the phototropic falls off more rapidly than the geotropic sensitivity. After 2 hours, however, a small part of the phototropic sensitivity is restored.

Discussion

As was already known from the literature, regeneration of auxin production, growth, and tropisms are closely related in decapitated Avena coleop-

tiles. In figure 3, all data from this paper on 5-mm. decapitated plants have been summarized, in all cases taking the intact plant as reference (100). The growth rate curve (B) is the smoothed curve derived from figure 1, corrected for the decreasing growth rate of the intact plants. The auxin content, curve A, is obtained from table I; the curve drawn between the points, which give the actual auxin content at 1, 2, 3, and 4 hours after decapitation, is purely arbitrary.

The auxin production curve, (C), is taken from figure 2. It should be drawn as a block diagram, but it has been smoothed for convenience. It shows that after 2 hours auxin is being produced, and this production continues at a constant or even increased rate. The curve for the geotropic sensitivity (D) is taken from the 5-mm. decapitated plants of table VII. The time of return of geotropic sensitivity lies somewhere near 120 minutes.

In comparing the 4 curves of figure 3, one fact stands out: curves A and B are very similar, as are curves C and D. From this figure it is evident that growth rate B and extractable auxin content A of coleoptiles parallel each other, with an increasing lag of the growth rate. This lag would be expected, since the growth response of coleoptile cells to auxin gradually decreases after decapitation (table II).

Curves C and D, which resemble A and B in their sudden increase around 120 minutes, are very different in other respects. Instead of slowly falling off in the first two hours, this drop is very sudden, and is even much more pronounced than in figure 3; for the actual *auxin* production in decapitated plants should be measured with the standard Avena test, and not with the deseeded test. Since only data for 2-hour auxin production are available with the standard Avena test (table III) no curve could be drawn with them, and the data obtained with the deseeded test have been substituted; these, as discussed before, are too high for the period before regeneration. The drop after three hours is not real, but occurs only in sections cut at the moment of decapitation. For in table VI it is shown that many hours after decapitation auxin production by the regenerated tip increases instead of decreases as in cut sections.

Curve D is also too high for the first 100 minutes, since the geotropic curvatures still appearing after decapitation occur only in the most basal zones. On the other hand, the curvatures of intact and regenerated decapitated plants are mainly in the more apical zones.

A number of conclusions can be drawn from this figure 3:

a) The sudden suspension of auxin production after decapitation is only gradually reflected in a decreased auxin content of the whole coleoptile, and soon after auxin production is resumed the auxin content of the tissues increases again. BONNER and THIMANN (**2**) have shown that the decrease in auxin content in the first two hours after decapitation corresponds quanti-

tatively with the amount of growth which has taken place in the coleoptile. Considering this quantitative correlation, and the similarity between extractable auxin content and growth rate, it cannot be doubted that the growth rate of the Avena coleoptile is regulated by the amount of *extractable* auxin in the tissues.

b) The growth rate of the decapitated coleoptile is *not* correlated with the amount of diffusible auxin; in other words, with the *rate* of auxin *production.*

c) The sudden drop in geotropic (and phototropic) response, to less than one half the initial value within 10 minutes after decapitation, cannot be accounted for by the drop in auxin content (probably 1 to 2 per cent. within the first 10 minutes).

It closely parallels the amount of diffusible auxin, however, and comes up as soon as auxin production is resumed in the regenerated physiological tip.

Consideration of all these facts leads to the main conclusion: Diffusible or free moving auxin and extractable auxin are quantitatively different in the plant; and as far as their effects go, also qualitatively. The diffusible auxin in the Avena coleoptile is correlated with tropisms. Since it has been concluded before that these are due to changes in the path of transport of auxins (CHOLODNY-WENT's theory) this might have been expected. Besides, since geotropism, and phototropism in part, are correlation phenomena, it is evident that they could be brought about only by a correlation-*carrier*, or moving agent; in other words, by free moving auxin.

The extractable auxin, comprising both free moving and bound auxin, is quite evidently *not* correlated with tropisms. But the growth rate closely parallels the extractable auxin; we must thus conclude that growth is due to the bound auxin. Therefore, the two methods of obtaining auxin, by diffusion and by extraction, are not interchangeable, and are not even comparable. This fact is brought out very clearly by VAN OVERBEEK (**11**) on completely different grounds.

Much confusion will be prevented by adapting the method of collecting and measuring auxin to the specific problem to be analyzed. If a correlation phenomenon is under investigation, it should be related to the diffusible auxin; growth rates should be compared with extractable auxin. This explains also why AVERY and LARUE (**1**) concluded that "Growth hormone secreted by the coleoptile tip has no helpful relationship to growth of coleoptiles in culture" for it is not the auxin formation but the auxin and precursor *content* which determines the growth rate. But it is equally clear that their other conclusion "that growth hormone is not a necessity for elongation of the coleoptile" does not follow from their experiments.

It is perhaps cogent to point out that the data presented in this paper very strongly oppose the suggestion made by SÖDING (**8**) and JOST (**5**) that auxin could be formed as a result of growth. Experimental evidence is overwhelmingly in favor of the conclusion that increased auxin content and growth follows increased auxin formation.

Since we must distinguish between two successive reactions in which auxin takes part, which have very different characteristics (**14**), and since we know that auxin is present in at least two different conditions inside the plant, it is interesting to attempt a correlation of these facts. In table VIII such an attempt is made. Thus far no facts are known which do not fit into this scheme. Some further remarks concerning each of the points of table VIII follow:

TABLE VIII

PROPERTIES OF AUXIN INSIDE THE AVENA COLEOPTILE

FREE MOVING AUXIN	BOUND AUXIN
1. Can be collected by diffusion out of the living organ into water (agar); its movement inside the plant is polar.	Extractable from the killed cells by organic solvents (alcohol, ether, chloroform, etc.)
2. It is derived from an auxin precursor.	Derived from the free moving auxin. Once it is bound it apparently is not converted into free moving auxin again.
3. Responsible for correlation phenomena such as tropisms, bud inhibition.	Responsible for visible cell elongation and root formation.
4. Not activated by light.	Partially inactivated by light.
5. Affects the first stages of growth and root formation, which are all due to the so-called preparatory reaction. This function can also be performed by hemi-auxins.	Causes the growth and root forming reactions proper.
6. Action not dependent on pH.	Reactive only at lower pH.
7. Double bond in molecule not essential for activity.	Only active with double bond in molecule.
8. Affects transport of other growth factors (calines).	Reacts with other growth factors (calines).
9. No stoichiometric relationship between chemically different free auxins and their effects has been pointed out as yet; probably does not combine with other compounds; otherwise it would not be free moving.	Stoichiometric relationship between bound auxin and growth suggests chemical combination with some other cell constituent.
10. Present in protoplasmic interphase?	Present inside protoplasm?

1. Is a description or definition.
2. This is the conclusion already reached (**15**).
3. Incorporates the conclusions from this paper, with additions (**14**).
4. Gives the conclusion reached by STEWART and WENT (**10**).
5. Gives the suggestion mentioned earlier in this paragraph. It is to some extent based on (3) and the discussion by WENT (**14**).
6. Follows from (5) and WENT (**14**).
7. In a paper by KOEPFLI and WENT to appear shortly it will be shown that the double bond in the auxin molecule is not essential for preparatory activity; some hemi-auxins do not have this double bond.

8, 9, and 10 are quite hypothetical; further discussion can be found in (**14**).

Summary

Avena coleoptiles were decapitated, and at suitable intervals, one-half to one hour, a number of their properties were measured. It was found that after decapitation the auxin content of the tissues, as determined by ether extraction, slowly fell off to 50 per cent. after 2 hours and then increased again. A similar curve was found for the growth rate, only the decrease was greater, especially after 2 hours. It was concluded that the growth rate is due to the amount of extractable auxin present in the tissues.

In the case of diffusible auxin from the various coleoptile zones, it was found that immediately after decapitation only small and decreasing amounts of precursor could be collected from any part of the coleoptile, and no auxin. But after 2 hours auxin production again became evident in the apical section. An almost identical behavior was found for geotropism and phototropism, a very abrupt falling off of geotropic sensitivity, which remained at a low level until approximately 2 hours after decapitation when it increased to about half normal. It was concluded that the geotropic curvature is due to an effect of gravity on the diffusible auxin.

This paper is also a complete confirmation of DOLK's thesis (**3**) that regeneration of the auxin production 2 to 3 hours after decapitation accounts for the reappearance of geo- and phototropic sensitivity and increased growth rate.

CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

LITERATURE CITED

1. AVERY, G. S., and LARUE, C. D. Growth and tropic responses of excised Avena coleoptiles in culture. Bot. Gaz. **100**: 186–199. 1938.
2. BONNER, J., and THIMANN, K. V. Studies on the growth hormone of plants. VII. The fate of growth substance in the plant and the

nature of the growth process. Jour. Gen. Physiol. **18**: 649–658. 1935.

3. DOLK, H. E. Concerning the sensitivity of decapitated coleoptiles of *Avena sativa* for light and gravitation. Proc. Kon. Akad. Wetensch. Amsterdam **29**: 1113–1117. 1926.
4. DUBUY, H. G. Über Wachstum und Phototropismus von *Avena sativa*. Rec. trav. bot. neérl. **30**: 798–925. 1933.
5. JOST, L. Zur Physiologie der Gefäszbildung. Zeitschr. Bot. **35**: 114–150. 1940.
6. LI, T–T. The appearance of the new physiological tip of the decapitated coleoptiles of *Avena sativa*. Proc. Kon. Akad. Wetensch. Amsterdam **33**: 1201–1205. 1930.
7. SCHNEIDER, C. L., and WENT, F. W. A photokymograph for the analysis of the Avena test. Bot. Gaz. **99**: 470–496. 1938.
8. SÖDING, H. Wuchsstoff und Kambiumtätigkeit der Bäume. Jahrb. wiss. Bot. **84**: 639–670. 1937.
9. SKOOG, F. A deseeded Avena test method for small amounts of auxin and auxin precursors. Jour. Gen. Physiol. **20**: 311–334. 1937.
10. STEWART, W. S., and WENT, F. W. Light stability of auxin in Avena coleoptiles. Bot. Gaz. **101**: 706–714. 1940.
11. VAN OVERBEEK, J. A quantitative study of auxin and its precursor in coleoptiles. Amer. Jour. Bot. **28**: 1–10. 1941.
12. WENT, F. W. Wuchsstoff und Wachstum. Rec. Trav. bot. neérl. **25**: 1–116. 1928.
13. ————. Remarks about two auxin problems. Chronica Botanica **4**: 503–505. 1938.
14. ————. Analysis and integration of various auxin effects. Proc. Kon. Ned. Akad. Wetensch. Amsterdam **42**: 581–591; 731–739. 1939.
15. ————, and THIMANN, K. V. Phytohormones. Macmillan. New York, 1937.

A NON-OSMOTIC FORCE IN THE WATER RELATIONS OF POTATO TUBERS DURING STORAGE

CHARLES J. LYON

Introduction

The potato tuber has long been used for routine demonstrations of osmosis in living tissues. Changes in turgor, weight, and volume have been brought about by suitable use of non-toxic compounds in aqueous solution and explained on the basis of diffusion pressures controlled by solutes, wall pressures, and differentially permeable membranes. On such qualitative evidence, supported to some extent by quantitative measurements, the water relations of the tissues are commonly regarded as dependent primarily upon osmosis.

In 1936 BENNET-CLARK, GREENWOOD, and BARKER (**2**) used a combination of plasmolytic and cryoscopic methods to obtain experimental evidence for the degree to which osmotic forces account for water movements in certain tissues with colored cell sap. In the petioles of Rheum and *Caladium bicolor* they found close agreement between the measurements of osmotic pressures by the two methods. In the roots of beet and swede (turnip) and in the petiole of *Begonia rex*, the measurements showed a consistently higher value for osmotic pressure as determined by plasmolysis. The difference was termed "secretion pressure" which was explained as a function of the protoplasm which appeared to secrete water from the external medium into the vacuole with a force that varied from two to six atmospheres.

This same discrepancy in measurements had been noted by BUHMANN (**3**) in 1935 but attributed to errors inherent in the usual method for determining the threshold of plasmolysis. When the minimum cell volume method was substituted for the separation of the cytoplasmic sac from the cell wall, there was no significant difference between the results of determinations by cryoscopic and plasmolytic methods.

Since BENNET-CLARK and his co-workers had found the unknown "pressure" in only a part of their plant tissues and had checked the finding by a second method of self-plasmolysis by expressed sap, the existence of a force which acts like inward secretion invites confirmation or a better interpretation. In their work with sap pressed from mesophyll cells in the cotton leaf, MASON and PHILLIS (**7**) found the osmotic pressure of vacuolar sap very much lower than that of the sucrose solution required to plasmolyze the cells. They considered their evidence as support for the suggested "secretion pressure" of the protoplasm which they also reported to contain a sap of its own with an osmotic pressure several times that of the vacuolar

sap. From work done by a still different method, it is now possible to report a similar non-osmotic force in potato tubers along with measurements of certain osmotic quantities in the same tissues.

Materials and methods

All measurements have been made by an extension of URSPRUNG's simplified method as described by LYON in 1936 (**5**), using the improvements suggested for the method in 1940 (**6**). The work was started in September, 1936, with several lots of tubers that were held in storage and tested at intervals during the fall and winter.

Before closing the tests in February, 1937, results of the final measurements of osmotic pressures were checked by determination of the freezing point depression, using a Beckman thermometer and tissue extracts removed by pressure from strips killed by freezing with solid carbon dioxide. In two cases the tissues were frozen immediately after taking the tuber from storage and removing a slice from one side (for use in the method being checked). In the third instance the tissue was frozen after immersion for four hours in a sucrose solution of the concentration which gave incipient plasmolysis by the method of minimum cell volume.

For each of these three check tests on tissue which had ceased to show a non-osmotic factor, as determined by the extension of URSPRUNG's method, there was reasonably close agreement between the values of total osmotic pressure indicated by the cryoscopic method and by the improved simplified method which was thereafter assumed to be sufficiently accurate for the purposes of the study. These check tests together with experience in measuring osmotic values on opposite sides of the same tuber by the simplified method alone, indicate errors in the latter up to 0.5 atm. for the mean net osmotic pressure of the cortical cells and 1.0 atm. for the total osmotic pressure of the cell contents.

The preliminary tests for changes in osmotic quantities of commercially grown tubers were repeated during the storage seasons of 1938–1939 and 1939–1940 with somewhat more exact measurements by the new method and with better storage conditions for the tubers. The tests were also continued into April of 1939 and May of 1940 when the cold storage tubers were badly sprouted. Because these two series of measurements were more precise and more complete without showing any significant differences in the period covered by the 1936–1937 tests, the results of the latter are not included here except as they can be said to support the conclusions drawn from the work done with tubers grown in 1938 and 1939.

The potatoes were grown from certified strains of the Green Mountain variety except for one lot of Early Rose in each season. The tubers were dug in the fields, selected for medium size, taken directly to the laboratory,

and each lot tested at once. The fields were scattered for a distance of about twenty-five miles along the Connecticut River valley, chiefly, on its terraces in New Hampshire. There were differences in the soils and fertilizers but no correlations have been noted between these factors and the behavior of the tubers in storage.

Each lot of potatoes was divided into two like portions. One was placed in a paper carton on a shelf in a refrigerator room regulated at 40 to 42° F. The other was stored in a covered cardboard box in a room held at 70° F. by day and about 65° F. at night.

All measurements were made in this room, using distilled water and a stock solution of sucrose kept sterile at room temperature. Each test required an interval of four hours between measurements of tissue strips before and after immersion in the graduated series of sugar solutions, controls being held in paraffin oil and measured twice in the same way. When the average length of the control strips changed (slightly), the second measurement was taken as the original length, since the same changes would take place in the strips immersed in sugar solutions for reasons unrelated to the movement of water in the tissues.

The solutions of sucrose, graded in steps appropriate to the conditions in the tubers at the time, were made by dilution of a volume molar stock solution. Calculations of osmotic pressures, based on the osmotic concentration within the tissue at incipient plasmolysis and the volumes of the tissue at the critical points of nearly plasmolyzed, normal and saturated with water, were made in terms of atmospheres at 20° C.

A specimen set of significant working data and calculations for a single test is given in table I. Three strips of tissue from scattered parts of a slice of one tuber were immersed in each of the several sterile sucrose solutions, in the dish of distilled water, and in the paraffin oil, all liquids being held in small covered dishes. The length of each strip was measured (without removal from the liquid) by means of a calibrated scale in one ocular of a wide-field dissecting binocular, carefully focused. The fully turgid condition in the distilled water gave the point of zero net osmotic pressure and the average length of tissue strip was recorded for this point of saturation with water. The length of strip as cut from the tuber appeared in the measurements of the tissue held in the oil bath. The solution of sucrose in which this "normal" condition was retained was found in the series of measurements or obtained by interpolation and recorded either by molar concentration or by the equivalent osmotic pressure in atmospheres. The osmotic pressure of the cell contents at the point of incipient plasmolysis was revealed by the most dilute solution in which the minimum length (and volume) of tissue strip appeared.

As shown by the example in section B of table I, the osmotic quantities

for each tuber tested were calculated from the data just described and use of the equation $N = C - W$ which states the relationship between net osmotic pressure of a cell (N), its wall pressure (W), and the theoretical osmotic pressure of its contents (C) as indicated by the measure of its solute concentration. These calculations are based on the well known HÖFLER scheme which in turn depends on the principles of: (1) inverse ratio between cell

TABLE I

SPECIMEN SET OF DATA AND CALCULATIONS

A. WORKING DATA FOR SINGLE TEST

CRITICAL POINT	CONCENTRATION OF SUCROSE	EQUIVALENT OSMOTIC PR.	AVERAGE LENGTH OF 3 TISSUE STRIPS
	M	*atm.*	*0.1-mm. units*
Incipient plasmolysis	0.70	21.5	59.5
Normal	0.32	8.7	61.9
Saturated	0	0	65.8

B. CALCULATIONS OF VALUES AT NORMAL CONDITION*

	INCIPIENT PLASMOLYSIS	NORMAL†	SATURATED
Length of strip	59.5	61.9	65.8
Volume " "	2106.0	2380	2860
C in atm.	21.5	X = 19.05	X′ = 15.88
W " "	0.0	Y = 5.77	X″ = 15.88
N " "	21.5	Z = 13.28	
		Observed N = 8.7	
		Discrepancy in N = 4.58	

* Formula: Net osmotic pressure = osmotic pressure of cell contents—wall pressure
$N = C - W$

† $X = \frac{21.5 \cdot 2106}{2380} = 19.05$

$X' = \frac{21.5 \cdot 2106}{2860} = 15.88$

} from inverse ratio between volume and solute concentration

$X'' = X'$ when $N = O$ in equation $N = C - W$

$Y = \frac{15.88\ (2380 - 2106)}{2860 - 2106} = 5.77$ (direct ratio between wall pressure and changes in cell volume)

$Z = X - Y = 19.05 - 5.77 = 13.28$ (calculated value of $N = C - W$)

Observed N = osmotic pressure of 0.32 M sol. = 8.7 atm. (from solution giving no change in volume of test strip)

Discrepancy in N (non-osmotic force) = 13.28 − 8.7 = 4.58 atm.

volume and the concentration of solutes within it; and (2), direct ratio between wall pressure and increase in volume from the fully flaccid to the fully turgid condition.

The volume of each strip was obtained from the measurement of its length by assuming that the original ratio of 1 to 10 between the width and length and the depth and length of each strip remained essentially un-

TABLE II

NORMAL OSMOTIC VALUES OF STORAGE POTATOES 1938–1939*

DATE	OSMOTIC PRESSURE OF CELL CONTENTS		TURGOR PRESSURE		CALCULATED NET OSMOTIC PRESSURE		OBSERVED NET OSMOTIC PRESSURE		NON-OSMOTIC FORCE	
	70° F.	42° F.	70° F.	42° F.	70° F.	42° F.	70° F.	42° F.	70° F.	42° F.
				ATHERTON GREEN MOUNTAIN						
	atm.	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*
Sept. 30	.....	13.00	.....	5.10	.....	7.9	.....	7.27	.....	0.63
Oct. 19	20.0	18.65	4.95	11.0	15.05	7.65	9.6	7.27	5.45	0.38
Nov. 19	.....	19.25	.....	9.5	.....	9.75	28.8	9.60	.....	0.15
Dec. 17	.....	24.45	.....	6.93	.....	17.52	19.6	11.10	.....	6.40
Jan. 19	.....	20.00	.....	3.32	.....	16.68	27.6	9.58	.....	7.10
Feb. 24	.....	19.93	.....	3.80	.....	16.13	34.6	9.58	.....	6.55
Mar. 25	.....	16.00	.....	9.40	.....	7.70		7.90	.....	0.20
				EATON GREEN MOUNTAIN						
Sept. 27	.....	13.0	.....	5.1	.....	7.9	.....	8.13	.....	0.23
Oct. 17	23.4	21.00	9.5	8.95	13.9	12.05	9.6	8.70	4.3	3.35
Nov. 17	32.9	22.25	6.2	8.95	26.7	13.30	19.6	13.15	7.1	0.15
Dec. 16	24.9	24.20	2.9	9.95	22.0	14.25	12.4	13.30	9.6	1.00
Jan. 18	.....	20.60	.....	3.20	.....	17.40	21.5	12.70	.....	4.70
Feb. 17	25.0	13.20	3.5	1.90	21.5	11.30	19.6	9.00	1.9	2.30
Mar. 16	.....	22.25	.....	8.10	.....	14.15	.....	8.70	.....	5.45
Apr. 15	.....	21.30	.....	12.30	.....	9.00	.....	6.15	.....	2.85

LYNES GREEN MOUNTAIN										
Sept. 27	.....	12.95	.....	5.1	.....	7.85	.....	6.7	.....	1.15
Oct. 16	21.80	21.45	8.4	8.62	13.4	12.83	8.42	5.3	5.00	7.53
Nov. 16	23.40	22.65	6.4	10.70	17.0	11.95	8.13	11.1	8.87	0.85
Dec. 15	.....	16.57	.....	5.85	.....	10.72	25.50	10.2	.....	0.52
Jan. 17	.....	17.42	.....	1.56	.....	15.86	20.70	11.1	.....	4.76
Feb. 16	.....	17.17	.....	2.70	.....	14.47	.....	11.9	.....	2.57
Mar. 16	.....	23.90	.....	6.20	.....	17.70	.....	8.7	.....	9.00
Apr. 15	.....	15.50	.....	7.30	.....	8.20	.....	6.7	.....	1.50
EARLY ROSE										
Sept. 15	.....	21.85	.....	9.75	.....	12.10	.....	8.43	.....	3.67
Oct. 10	32.30	21.86	11.75	8.91	20.55	12.95	17.80	8.10	2.75	4.85
Nov. 3	23.68	19.12	5.12	13.75	18.56	5.37	16.70	5.30	1.86	0.07
Dec. 4	25.00	13.85	2.19	2.55	22.80	11.30	11.70	10.80	11.10	0.50
Jan. 2	.....	16.47	.....	5.17	.....	11.30	21.50	11.10	.....	0.20
Feb. 2	.....	16.94	.....	2.41	.....	14.53	32.10	10.20	.....	4.33
Mar. 3	.....	21.00	.....	3.67	.....	17.33	.....	9.58	.....	7.75
Apr. 3	.....	15.90	.....	2.50	.....	13.40	.....	9.30	.....	4.10

* Data tied together by braces were obtained from measurements of the same tuber.

TABLE III

NORMAL OSMOTIC VALUES OF STORAGE POTATOES 1939–1940*

DATE	OSMOTIC PRESSURE OF CELL CONTENTS		TURGOR PRESSURE		CALCULATED NET OSMOTIC PRESSURE		OBSERVED NET OSMOTIC PRESSURE		NON-OSMOTIC FORCE	
	70° F.	42° F.	70° F.	42° F.	70° F.	42° F.	70° F.	42° F.	70° F.	42° F.
ATHERTON GREEN MOUNTAIN										
	atm.	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*	*atm.*
Sept. 14	.....	8.4	.....	2.55	.....	5.85	....	6.7	.....	0.85
Oct. 5	11.4	15.80	2.40	6.75	9.00	9.05	5.3	9.58	3.70	0.53
Nov. 7	19.0	12.60	4.20	3.90	14.80	8.70	9.6	8.10	5.20	0.60
Dec. 7	22.4	15.70	5.00	6.70	17.40	9.00	8.7	9.00	8.70	0.00
Jan. 7	22.0	16.35	4.50	3.23	17.50	13.12	8.4	9.00	9.10	4.12
Feb. 6	19.1	16.00	8.20	4.75	10.90	11.25	8.1	8.70	2.80	2.55
Mar. 6	.....	18.95	.....	8.25	.....	10.70	....	8.60	.....	2.10
Apr. 3	.....	18.70	.....	9.60	.....	9.10	....	8.40	.....	0.70
Apr. 5	.....	18.50	.....	4.05	.....	14.45	....	5.30	.....	9.15
May 14	.....	23.10	.....	3.25	.....	19.85	....	8.00	.....	11.85
BROWN GREEN MOUNTAIN										
Sept. 15	.....	9.5	.....	3.90	.....	5.60	.....	6.2	.....	0.60
Oct. 9	15.5	12.60	6.70	3.40	8.80	9.20	6.1	9.60	2.70	0.40
Nov. 11	15.6	21.45	3.70	11.05	11.90	10.40	7.1	9.90	4.80	0.50
Dec. 8	15.7	15.80	3.50	4.70	12.20	11.10	5.9	9.60	6.30	1.50
Jan. 8	16.0	15.30	3.80	4.05	12.20	11.25	5.3	8.10	6.90	3.15
Feb. 7	19.1	16.05	5.90	2.30	13.20	13.75	6.4	7.85	6.80	5.90
Mar. 7	24.5	15.00	2.15	3.40	22.35	11.60	11.7	8.70	10.55	2.90
Apr. 3	.....	18.80	.....	7.55	.....	11.25	.....	8.60	.....	2.65
May 14	.....	19.30	.....	5.00	.....	14.30	.....	7.80	.....	6.50
DENSMORE GREEN MOUNTAIN										
Sept. 15	.....	10.25	.....	3.95	.....	6.30	.....	6.30	.....	0.00
Oct. 12	16.10	15.80	4.00	3.14	12.10	12.66	6.40	12.70	5.70	0.00
Nov. 12	21.50	21.10	6.50	9.80	15.00	11.30	7.80	11.10	7.20	0.20
Dec. 12	22.00	15.60	5.60	4.45	16.40	11.15	9.60	8.15	6.80	3.00
Jan. 10	20.00	18.90	3.45	6.30	16.55	12.60	9.30	8.10	7.25	4.50
Feb. 8	16.30	22.40	3.50	6.15	12.80	16.25	6.15	9.00	6.65	7.25
Mar. 6	.....	19.10	.....	5.77	.....	13.33	.....	8.70	.....	4.63
Apr. 4	.....	18.55	.....	4.05	.....	14.50	.....	6.70	.....	7.80
May 14	.....	11.05	.....	3.10	.....	7.95	.....	4.75	.....	3.20

May 14	.....	11.05	.....	3.10	.....	7.95	.....	4.75	.....	3.20

Lynes Green Mountain

Sept. 16	.....	10.20	.....	3.15	.....	7.05	.....	6.4	.....	0.65
Oct. 13	18.55	17.90	2.75	6.65	15.80	11.25	8.1	9.60	7.70	1.65
Nov. 13	22.35	21.15	4.30	8.90	18.05	12.25	8.7	8.70	9.35	3.55
Dec. 13	16.15	21.50	2.40	5.50	13.75	16.00	6.7	6.15	7.05	9.85
Jan. 11	16.15	18.90	2.30	7.10	13.85	11.80	8.4	8.10	5.45	3.70
Feb. 9	26.60	22.60	4.10	5.60	22.50	17.00	8.4	9.30	14.10	7.70
Mar. 9	.....	14.00	.....	4.70	.....	9.30	.....	4.80	.....	4.50
Apr. 4	.....	21.60	.....	7.60	.....	14.00	.....	6.15	.....	7.85
May 15	.....	20.60	.....	8.30	.....	12.30	.....	6.70	.....	5.60

Early Rose

Sept. 16	.....	9.80	.....	3.10	.....	6.7	.....	6.45	.....	0.25
Oct. 17	19.00	18.50	4.70	6.90	14.3	11.6	9.60	9.60	4.7	2.00
Nov. 16	16.70	19.45	3.50	6.15	13.2	13.3	12.70	13.00	0.5	0.30
Dec. 14	16.95	16.00	3.35	4.90	13.6	11.1	11.1	11.10	2.5	0.00
Jan. 12	13.70	16.50	2.70	3.80	11.0	12.7	9.30	9.00	1.7	3.70
Feb. 10	15.90	15.00	3.80	4.50	12.1	10.5	9.30	5.60	2.8	4.90
Mar. 9	.....	14.90	.....	3.40	.....	11.5	.....	8.10	.....	3.40
Apr. 5	.....	15.30	.....	4.20	.....	11.1	.....	7.50	.....	3.60
May 14	.....	15.95	.....	5.55	.....	10.4	.....	7.55	.....	2.85

* Data tied together by braces were obtained from measurements of the same tuber.

changed for the liquids in which the strips were immersed. All tests of this assumption have confirmed its accuracy for the tissues used in this work. For this and other details of the methods used, the original descriptions of the methods should be consulted (**5, 6**).

Results and discussion

The stored tubers of the 1938 and 1939 crops were tested at monthly intervals from the day they were dug until they had grown long sprouts. For no apparent reason, they kept better in both warm and cold storage during the second of these series although the rooms, temperatures, and even the cardboard containers were identical. Two lots of each season were grown by the same farmers in the same fields, with essentially the same fertilizers, yet the keeping qualities of the same strain of potatoes differed as greatly as the other lots.

The results of the tests and computations of osmotic quantities cannot be presented in a generalized form because each of the nine lots of tubers had its own values and characteristic behavior. From table I it will be evident that the essential data have to do with the "normal" conditions of the tissue, rather than the osmotic quantities at incipient plasmolysis or at maximum turgor. The extent of cell shrinkage and expansion at these two critical points is probably significant but some effects of them appear in the calculated normal values. These data are given in table II and III where the results for each lot of tubers are arranged to show the changes and variations during the storage season. The proper name attached to each sample of the Green Mountain variety indicates the grower. For each lot the first entry shows the osmotic values as the tubers came from the soil, measured as rapidly as possible, and entered in the 42° column.

Changes in osmotic quantities

The nine series of data for tubers stored under favorable and rather unfavorable conditions reveal some curious fluctuations in osmotic quantities along with a few well defined tendencies. For this part of the study the results are primarily a survey of changing forces, indicating a need for a parallel chemical analysis to identify the principal osmotically active substances. Certain conditions can be inferred, however, from the changes in total osmotic pressures, wall pressures, and net osmotic pressures, all computed or measured for the "normal" condition of the tubers.

The total force in the cells that is measured as osmotic pressure increases sharply during the month after the tuber is dug. For the cold storage, this is easily understood on the basis of the sugar that appears quickly in storage at 41° F. (**1**), but the same worker found no increase in sugar during storage at even 59° F. until several months had passed. Various other workers in-

cluding WRIGHT (9) have established the production of sugar at low temperatures only. At 70° F. no rise in sugar content can be expected and there is no other obvious source for a great increase in solutes; the increase in apparent "osmotic" pressure, however, continues with little or no loss during the month or two before the tuber becomes so flabby that the strips of tissue cannot be cut accurately for test purposes. In cold storage the gain in apparent osmotic pressure of the cell contents is usually lost, to some extent, in mid-winter but it returns in the spring, either temporarily, as in March of 1939, or for several months as in 1940. The loss in winter is probably something more than materials consumed by respiration because the loss is relatively small in warm storage. Changes in the cell contents may be in total concentration of solutes but the method of measurement does not preclude other controls of water movement.

The behavior of the wall pressure and opposing turgor pressure was not in accord with the idea of gradual loss of turgor as a tuber loses water by evaporation. In 1938 the turgor pressures at harvest time were consistently higher than those of 1939, in agreement with the moisture content of the soils at the time. As a rule, the calculated turgor rose considerably in both warm and cold storage but by early winter it had fallen again. In the poor-keeping season of 1938–1939, it remained down in warm storage while curiously rising again in cold storage; in several instances, however, the supposed increase of turgor was not apparent in the physical appearance of the tuber.

After the winter drop in the season of 1939–1940, the calculated turgor pressure usually went up again in both warm and cold storage, but not up so much then as did the total osmotic pressure. It seemed to follow the latter and yet show water losses at the same time. The apparent increase of calculated turgor in warm storage was notable and may be related to the excellent keeping qualities of the 1939 potatoes. Each lot of tubers in warm storage was subject to some loss of water to the dry air of the room when the box was opened each month but this loss was not great because the small box was tightly covered at other times. No water was supplied, however, and the apparent increase in turgor may have been unreal.

With all these changes in calculated wall pressure, it must be kept in mind that a non-osmotic force has been demonstrated simultaneously. Since it affects the calculations of normal "osmotic" values by including the results of other forces throughout the data, it is doubtful if valid measurements of wall pressures can be had by the method *in the presence of the unknown force*. It may be possible to have a seemingly greater turgor pressure even when the tuber is less firm to the touch than before.

On the contrary, there is some evidence from the direct measurement of net osmotic pressure that the turgor pressure may really increase late in

storage, since the net osmotic pressure becomes appreciably less at the same time. The data for 1938 Green Mountain potatoes in cold storage (table II) show examples of such unexpected changes. During the second month of their storage, a marked increase in the net osmotic pressure appeared and remained in force during the winter but most of it had disappeared by April, 1939. The Early Rose of the 1938 crop acted essentially the same in cold storage. Metabolic water is a possible source for lowering the net osmotic pressure and increasing the turgor and wall pressures; of course the net osmotic pressure can decrease by a change in solute concentration within the cells.

The evidence from the 1939 tubers in cold storage is not so exact on the point but the tendency is the same. The range of their net osmotic pressures is less and there are some irregularities in the trends, yet the final measurement of each series is well below the maximum N reached early in storage, and below the mean value for late fall and winter. The net osmotic pressure, as measured directly by the solution in which no change in cell volumes occur, need not be high after refrigerator storage. However, the 1939–1940 tubers in cold storage usually did not show the corresponding terminal increase in turgor pressure.

In warm storage the observed net osmotic pressure behaved as one would predict, from knowledge of inevitable water losses to a dry air, only for the 1938 tubers. Their observed N's increased rather evenly to a high value. Under identical conditions the 1939 crop rarely showed a rise in observed net osmotic pressure of more than two or three atmospheres, and this chiefly with the Early Rose variety that held the rise only two months. From the data in table III, it is evident that for this well known osmotic quantity the tubers were no better off in cold storage than in a warm room and often less so. Yet the warm tubers always collapsed first. Water relations and "keeping" qualities have curious and complex connotations.

The trends in the calcuated net osmotic pressures cannot be described so easily. They are irregular although certain tendencies can be seen in a majority of the lots measured each year. There is evidence for a decreased shortage of water at the end of cold storage for both 1938 and 1939 crops, as measured by lower net osmotic pressures in the final tests for seven lots out of nine. The calculated N's, however, probably tell more about the non-osmotic force than they prove for osmotic relations; *e.g.*, the seven smaller values at the ends of a series of calculated net osmotic pressures also indicate a closer agreement of calculated and observed N's then and a partial disappearance of the unknown force, since the calculated N's drop much faster than the corresponding observed N's.

The calculated net osmotic pressures of tubers in cold storage follow the general pattern of going up, down, up again, and usually down at the end

as just described. The first decrease in magnitude usually appears in February or March, or somewhat earlier in the 1938–1939 storage season. It could be interpreted as a loss in sugars through respiration, after which more solutes are formed as the chemical changes within the tuber form the first stages of the growth process, that even cold storage does not prevent entirely. Since all these data are calculated, however, such an interpretation is valid only if osmosis is the dominant force in the water relations of the tissues.

In warm storage the changes in calculated net osmotic pressure appear earlier and are greater in amount, but the pattern is not clear because of poor agreement among the nine lots of tubers. In six cases there is a decrease in the value of the calculated N, either just previous to the collapse or showing in the test just before the last one. As in the case of the total osmotic pressure, however, the decreases are smaller than the ones appearing in cold storage tubers of the same sample and are therefore not clearly attributable to respiration. There seems to be in these data a resultant of the effects of respiration, evaporation, and the non-osmotic force that appears in the calculated N's because it entered the calculations at various points.

The non-osmotic force

The existence of this factor in the water relations of potato tissue, suggested by unpredictable variations in the osmotic quantities of cells in their normal state, is demonstrated and to a certain degree measured by the discrepancy between the calculated net osmotic pressure and the observed value of it. With very few exceptions the difference is always in favor of the calculated amount. The discrepancy varies from 0 to 11.85 atmospheres for cold storage and to 14.1 for warm storage. If it were inherent in the method used in this study, it should always appear and its magnitude should be roughly proportional to that of some osmotic quantity that dominates the computations, such as total osmotic pressure of the cell contents. No such relation can be seen and at certain periods there is close agreement between the two values for N.

The discrepancy in the data for the net osmotic pressure of the tubers is more than variable—it behaves in a characteristic way. At harvest time it fluctuates within a narrow range either side of zero, with only one exception. In cold storage it increases, either slowly or within the first month, by a value of several atmospheres. In warm storage the increase is greater, always appears within the first month, and usually remains at that or a somewhat higher level as long as the tuber lasts; the only important exception is shown by the Early Rose variety.

Some time after the preliminary increase is reached in cold storage, commonly in late fall or early winter and always appearing somewhere in eight

out of the nine lots of tubers, there is a drop of several atmospheres in the discrepancy. It is frequently shown in the second of two measurements with the same tuber, as in the EATON lot of table II and the ATHERTON lot of table III. Since the magnitude of the discrepancy depends upon the trends in the two values for net osmotic pressure at the time (calculated and observed), it is interesting to note from the data in the tables that the drop usually means a lower calculated N rather than a higher observed N. This in turn means a different behavior of the tissue in the series of sugar solutions, with particular reference to the amount of shrinkage and swelling. The less the shrinkage in the solution giving incipient plasmolysis and the greater the swelling in distilled water, the greater will be the discrepancy between calculated and observed net osmotic pressure. A smaller discrepancy means a partial disappearance of the non-osmotic force.

Following the lower winter value of the discrepancy in N's and therefore of the non-osmotic control of the diffusion pressure of water within the tissues, there is always another increase which is usually lost in the final measurement of each series, when the magnitude of the discrepancy falls sharply. At this time the tuber has at least short sprouts and a partial flabbiness that is something more than a loss of turgor or rise in net osmotic pressure, both of which often go in the opposing direction at this time.

For several reasons this difference in net osmotic pressure demonstrates the existence of a non-osmotic force in the stored potatoes. Some of them have been mentioned in the discussion of trends in osmotic values during storage when the indicated trends were shown to be illogical. One example of this is the apparent loss of osmotically active substances in mid-winter cold storage but with less apparent loss in the more actively respiring tubers held in warm storage. The curious changes in turgor pressure and net osmotic pressure were also noted as difficult to understand on a purely osmotic basis.

The best defined proof that the discrepancy in N's means a non-osmotic force comes from the logic of the situation. If the force that determined the measure of osmotic concentration in the cells at the point of incipient plasmolysis were completely osmotic, the cells should obey the corresponding laws of physical chemistry when immersed in the sugar solutions and in the pure water during the monthly tests of the tissue. Specifically they should so shrink below the point of no change in volume in the isotonic solution and so swell in the pure water as to permit a reasonably accurate calculation of the net osmotic pressure in the normal condition, the osmotic quantity that can be measured most accurately for comparison. The discrepancy must be caused by conflict between osmosis and some other force that influences the diffusion pressure of the water in and about the cells.

Perhaps this origin of the discrepancy can be appreciated better by ref-

erence to the specimen set of data and calculations in table I. The minimum length of strip showed a shrinkage of only 3.9 per cent. (6.19 to 5.95 mm.) from that as the tissue was removed from the tuber's cortex, but this meant a decrease in volume of 11.5 per cent. When strips of the same original length and source were immersed in water, the volume of each increased by 20.2 per cent. (2380 to 2860 cubic units) until the turgor pressure reached its maximum and there was no net osmotic pressure. Computations showed these quantities to have been respectively 5.77 atm. and 13.28 atm. when the volume of each strip was 2380 units. Since the observed net osmotic pressure was only 8.7 atm. at this volume, the discrepancy in N's was well beyond any experimental error.

This large discrepancy is certainly brought about by the calculated N for the normal condition being too great, since the observed value of it is not subject to appreciable errors. There are two principal ways by which the calculated N can become too large: the calculated normal wall pressure may be too small, or the osmotic pressure of the cell content (C) may be calculated as too great. The latter could occur only if the strips had not shrunk enough from normal condition to the stage of incipient plasmolysis. Small errors in this matter are possible but a simple calculation will show that a total volume shrinkage of 25 per cent. (rather than 11.5 per cent.) would be needed to provide in this case a calculated net osmotic pressure equal to that of the observed N, the swelling in water to remain the same. Such an error is unlikely as a regular occurrence and there is no apparent method by which the elastic cell walls of the potato tissue could be conditioned against return to correct minimum lengths when the turgor pressure is reduced to zero by withdrawal of water.

It is more likely that a non-osmotic factor within the cells caused them to swell so much in pure water that the wall pressure in the normal condition, calculated on the assumption of osmosis as the only factor important to the diffusion pressure of water, appeared between 4 and 5 atmospheres too small. This "extra" swelling can be estimated by trial and error methods. In this case if the average length of the strips immersed in water had been 6.37 mm. (rather than 6.58 mm.) the calculated wall pressures would have been 10.15 atm. and the calculated net osmotic pressure would have been 8.9 atm. This is very close to the observed value of 8.7 atm. By osmosis alone, therefore, the cells should have swelled only about 8.4 per cent. by volume rather than the 20.2 per cent. they did. The difference of nearly 12 per cent. explains the discrepancy between the two values obtained for net osmotic pressure in this typical case of some non-osmotic force acting along with osmosis.

If this type of "extra" swelling in distilled water appeared only after the tubers had been in storage for some time under favorable conditions or for a shorter period with less favorable conditions, it would be possible to explain the excess swelling as a consequence of weakened cell walls. Two

characteristics of the data in tables II and III make this simple explanation unreasonable. The frequency with which the non-osmotic force appears in early autumn when the tubers are held in adequate cold storage suggests it is not an illusion caused by failing cell walls. Fairly good proof of the point is furnished later in each cold storage series when the magnitude of the non-osmotic force is seen to vary greatly, often within the same tuber, and especially when it becomes small or smaller in tubers six or eight months in storage and rapidly breaking down as the sprouts develop. It appears from this that the non-osmotic force varies in the manner already described and often becomes less noticeable after prolonged cold storage, unless the further assumption is made that cell walls can weaken and later recover much of their original strength or quality. There seems to be no basis for this belief.

With some non-osmotic factor in force and often varying in intensity, we have much the same situation that BENNET-CLARK and his colleagues found in some of their plant tissues. They pictured the second force as a vital activity of the cytoplasm about the cell vacuole, using energy supplied by metabolism. This interpretation may be accurate but the proof of it is incomplete. The force could be one of pure chemistry, such as an effect of colloids on the diffusion pressure of water within the cells or a difference in electrical potentials, either of which could vary during storage through physiological changes.

There is one other line of evidence which indicates some close connection between the living cells of potato tubers and a force which affects osmotic measurements of the cells concerned. When the total osmotic pressure is calculated from freezing point depression, the value obtained by inserting a thermocouple into the living tuber and freezing the intact tissue is higher than that obtained from freezing tissue extracts. The point was thoroughly studied by WALTER and WEISMAN (**8**) who attributed the differences to accumulated errors in working with living cells. Similar conclusions were reached by ZIMPFER and MEYER from results as yet unpublished (**10**). They found a good agreement between values from expressed sap and those from thermocouple measurements in dead, intact tissue. Before the tuber was killed the thermocouple method had indicated an osmotic pressure about twice that recorded after the cells were dead.

Other workers have noted this apparent drop in osmotic pressure in other tissues upon death but there has usually been some doubt of the accuracy of the determinations before death, always assuming that an accurate measure of solute concentration was sought. An effect of the "cellular membrane" is suggested by LUYET and GEHENIO (**4**) who found a lower freezing point in the living tissue of potato tuber but not of a myxomycete which has no cellular structure in the stage used for the experiments.

It is probably advisable to measure true osmotic pressures outside of

living cells but the proof that other methods are invalid for the determination of solute concentrations does not affect the existence of a non-osmotic force as a factor in the water relations of certain living tissues. At least part of the supposedly large "error" in the thermocouple method for living potato tuber, for example, could well be the same non-osmotic force that has appeared in the tubers used in this study, demonstrated by an entirely different method. It may add considerably to the frost resistance of tissues that possess it. The work of BENNET-CLARK and others has indicated this force in a variety of tissues. It is a strong enough force to be important in the water relations of the plants concerned. If it depends on some property of cell walls or cytoplasmic membranes, it is still important because they are characteristic of most plant tissues. Until more evidence is supplied, perhaps by closer study of the chemical changes during storage, the basis for the non-osmotic force in stored potato tubers cannot be identified but its existence can hardly be denied.

Summary

The osmotic quantities of potato tubers were measured at monthly intervals during two storage seasons, using an extension of the HÖFLER-URSPRUNG method for single cells but modified for work with blocks of homogeneous tissues under low magnification. The measurements were started with freshly dug tubers and continued until they collapsed in warm storage and sprouted in cold storage. The osmotic quantities for the "normal" condition of the tissues were computed or determined directly. Total osmotic pressure apparently increased soon after storage was begun but fluctuated in characteristic ways during the winter and spring. During storage the turgor pressure showed some effect of water lost by evaporation but seemed to depend more on the apparent total osmotic pressures of the cells. It often increased toward the end of the storage season, particularly in the tubers that showed the best keeping qualities. As measured by the sucrose solution in which the volume of the tissue was unchanged, the net osmotic pressure in cold storage usually increased during the winter but became smaller in early spring. In warm storage it was higher than in cold storage only if the tubers did not keep well. The calculated net osmotic pressures were usually significantly larger than the observed values. The discrepancy is a demonstration and approximate measure of a non-osmotic force. It appears in early storage and varies in size with the season and the storage temperature. It is comparable to the "secretion pressure" reported by BENNET-CLARK and others and to the excess "osmotic" pressure in living tissues over that in the same dead cells, as measured by the thermocouple method for freezing point depression; in potatoes, however, the basis for it is unidentified.

DARTMOUTH COLLEGE
HANOVER, N. H.

LITERATURE CITED

1. Barker, J. Changes of sugar-content and respiration in potatoes stored at different temperatures. Rep. Food Invest. Bd. Great Britain **1937**: 175–177. 1938.
2. Bennet-Clark, T. A., Greenwood, A. D., and Barker, J. W. Water relations and osmotic pressures of plant cells. New Phyt. **35**: 277–291. 1936.
3. Buhmann, Annie. Kritische Untersuchungen über vergleichende plasmolytische und kryoskopische Bestimmungen des osmotischen Wertes bei Pflanzen. Protoplasma **23**: 579–612. 1935.
4. Luyet, B. J., and Gehenio, P. M. Life and death at low temperatures. Biodynamica, Normandy, Missouri. Page 123. 1940.
5. Lyon, C. J. Analysis of osmotic relations by extending the simplified method. Plant Physiol. **11**: 167–172. 1936.
6. ————. Improvements in the simplified method for osmotic measurements. Plant Physiol. **15**: 561–562. 1940.
7. Mason, T. G., and Phillis, E. Experiments on the extraction of sap from the vacuole of the leaf of the cotton plant and their bearing on the osmotic theory of water absorption by the cell. Ann. Bot. N.S. **3**: 531–544. 1939.
8. Walter, H., and Weismann, O. Über die Gefrierpunkte und osmotischen Werte lebender und toter pflanzlicher Gewebe. Jahrb. wiss. Bot. **82**: 273–310. 1935.
9. Wright, R. C. Some physiological studies of potatoes in storage. Jour. Agr. Res. **45**: 543–555. 1932.
10. Zimpfer, P., and Meyer, B. S. Personal letter from Dr. Meyer. 1940.

STUDY OF RESPIRATION IN HEALTHY AND MOSAIC-INFECTED TOBACCO PLANTS

VIOLETTE F. C.-GLASSTONE

(WITH FIVE FIGURES)

Introduction

Comparatively little investigation has been made into the fundamental metabolic processes of plant viruses. BRONFENBRENNER and REICHERT (**2, 3, 4**) were unable to detect any respiration in either viruses or bacteriophages. The isolation of virus, free from plant cells, and in a condition suitable for such experiments, is obviously very difficult. The effect of virus infections on the metabolism of host plants is also a matter of great interest and may help to elucidate the action of the virus if sufficient data are collected. Since most viruses affect the chlorophyll content of the plant, it is not surprising that the work that has been done so far has been directed primarily on respiration. THUNG (**20**) observed that the respiration rate of potato leaves infected with leafroll was higher than that of the corresponding healthy leaves. Also working with potatoes infected by leafroll, WHITEHEAD (**21**) studied the course of respiration from the immature tuber up to the development of the plant. In maturing tubers and in leaves, or shoots with expanded leaves, the respiration rate of the diseased plants was higher than that of the healthy plants; but for a period lasting from dormancy of the tuber until the expansion of the leaves, the respiration rate of the healthy material was the greater. This was true both for aerobic and anaerobic conditions, and also for various conditions of light, temperature, and carbon dioxide supply. He concluded that the virus affects the respiration rate not directly, but only by interfering with the translocation of the respirable substrate.

In a number of species of plants naturally infected by mosaic or yellows virus diseases it was found (**9**) that the respiration rate was increased in young leaves affected by virus but was decreased in older leaves thus affected. This was not apparently connected with the variations in carbohydrate or nitrogen contents of the leaves. On the other hand, DUFRÉNOY (**8**) noted that the high rate of respiration in healthy tobacco buds was decreased by tobacco-mosaic virus, whereas the lower respiratory activity of the discolored leaves was increased by the presence of the virus.

CALDWELL (**6**) inoculated tomato plants with aucuba mosaic as seedlings and at the five-leaf stage and measured the respiration of the diseased tops. In all his experiments there was an initial high output of carbon dioxide which fell off to a steady level. Tops of plants inoculated at the five-leaf stage evolved more carbon dioxide than the healthy controls throughout the

experimental period; but those inoculated as seedlings showed a lower output at the beginning, followed by a higher output than the healthy tops once the stable level had been reached. He considered his experiments indicated that the virus affects the enzymes which "activate" the substrate before it is broken down to carbon dioxide in the respiration mechanism.

In a series of experiments on discs of healthy and tobacco-mosaic-infected tobacco leaves carried out by LEMMON (**17**), the respiration rate of the healthy leaves was always higher than that of the diseased. KEMPNER (**15**) was unable to find any change in the respiration of tobacco leaves infected by tobacco-mosaic virus, unless necrosis had set in.

There appears therefore to be a certain amount of discrepancy among the published results of the various workers in this field. All the experiments quoted above were made with tissue that was already diseased, and it seemed possible that a study covering the entire development of the disease would be profitable.

During the summer of 1940 the writer made some preliminary observations on the respiration of plants grown in soil. A respiration chamber was sealed over the plant top by means of a wax mixture. This seal was perfectly airtight, and it is usual to assume that the respiration of the top can be measured independently from that of the root by such a device. It was discovered, however, that air can pass easily through plants, especially tobacco, from root to leaf and *vice versa* (**10**). Hence it was possible that the air in the soil or roots below the surface of the wax seal was in some way interfering with the measurements.

For this reason it was decided to experiment with entire plants in nutrient solution to avoid effects due to the passage of air from other organs or to cutting, handling, drying out, or partial starvation of isolated material, all of which are known to have some influence on respiration. The present paper therefore is a report of experiments designed to follow the course of the respiration in entire plants from the time of their inoculation up to the appearance of the mosaic mottling, using the same plants throughout the period. The work was done in the spring of 1941.

Material

Ordinary tobacco-mosaic virus was used on *Nicotiana tabacum* L. var. Samsun as the host plant. This proved to be a very suitable subject for the purpose, as the resulting disease did not distort the plant unduly, merely producing a reduction of chlorophyll in a mottled pattern and causing stunting. There was also the advantage that under favorable conditions of light and temperature it was possible to note the time that the disease became systemic by observing the "clearing of the veins" (**13**).

The seeds were sown in nutrient sand, and as soon as the plants were

large enough to handle they were transferred to nutrient solution. The solution recommended by HOAGLAND and ARNON (11) with rather more ferric sulphate was found very satisfactory. The nutrient solution was contained in 4-liter battery jars which were covered with thick black paper to prevent growth of algae on the roots: no algae formed during any experiment. The plants were supported on thick wooden discs, containing holes for the roots. The discs were well waxed with paraffin wax, which was renewed for each set of experiments. Great care was taken to keep separate all the glassware and discs for the healthy and diseased plants, in order to avoid contamination. The only contaminations, which will be noted in the results, were due to an accident, the origin of which was later discovered. The younger plants stood in the wooden discs without further support; the older plants were usually held in place by cotton wads or occasionally by glass rods. The solution was changed twice to four times a week, according to the quantity of plant material. The plants ranged in size from the smallest that could be handled conveniently, of an average individual weight of 0.5 gm., up to the largest that could be put in the respiration chambers, of an average weight of 40 gm. In any case it was considered desirable for this experiment to avoid plants forming flowers, so the range in size was actually the widest that could have been used under the circumstances.

The diseased plants were inoculated in the usual manner by rubbing with a muslin pad dipped in tobacco-mosaic-virus juice (12). The healthy control plants were rubbed with a similar pad dipped in distilled water to equalize the treatment as far as possible. Plants were thus inoculated after being in nutrient solution for periods of 1 day, 1 week, 2 weeks, 3 weeks, and 4 weeks, respectively. The measurements of respiration rate were made on groups of plants from the first day of inoculation up to the time that the mosaic mottling developed. Two weeks were allowed for each group, which was usually long enough. The experiments in nutrient solution were repeated at least once for each time period.

As the respiration measurements were carried out in the daytime, the plants were illuminated artificially at night by means of 1000-watt Mazda bulbs. As a check on the growth and development of the virus, a group of plants in soil grown under normal greenhouse conditions was always used as observational controls. It was found that the clearing of the veins and mottling always appeared on the same days in both the greenhouse plants and in those receiving artificial illumination. The weight measurements were made daily on the same plants by weighing the entire plant. The plants were removed singly from the nutrient solution, placed carefully on a soft tissue, and the roots were gently blotted with another tissue to remove the moisture. The plant was then quickly weighed and replaced in solution. After a little practice, the whole process of weighing could be performed

very rapidly with no apparent injury to the plants. In figure 1 are shown two plants which were weighed in this manner for 50 days. The plant on the left was the healthy control and the other was diseased. It will be seen that the roots as well as the rest of the plants are in good condition.

Method

The apparatus consisted of 6 units, one of which is shown diagrammatically in figure 2. Air from a compressed air manifold was freed from carbon dioxide by passage through a soda-lime tower, A, a sodium hydroxide

FIG. 1. Plants photographed after 50 days of experimentation. (Photograph by J. A. CARLILE.)

solution, B, and a dilute barium hydroxide solution tinted with phenolphthalein, C. The latter also acted as an indicator trap. The carbon-dioxide-free air passed through a flowmeter, D, provided with its own overflow trap, and thence via a tube, J, into the respiration chamber, E. This consisted of a large bell jar, 53 cm. high and 26 cm. internal diameter, sealed firmly to a base of ground plate glass, F, by means of a ring of plasticum clay, G, and enclosed during the experiment with a cover of thick black satin to exclude

all light. The air next passed through the tube, K,K, to enter the titration flasks, P (a very slight modification of that used by MACK, **18**). The exit tube, Q, from this flask led to a glass tower, R, 65 cm. long, containing glass beads; next, by a glass tube, S, to an indicator trap, T, containing dilute barium hydroxide solution, and thence to the vacuum manifold. With the exception of the brass manifolds, glass tubing, joined by butt to butt joints in thick rubber pressure tubing, was used throughout. The 6 units were connected by the manifolds leading from the compressed air and vacuum mains. Each unit had its own taps to the 2 manifolds, and the vacuum manifold was further protected from sudden changes of pressure by mer-

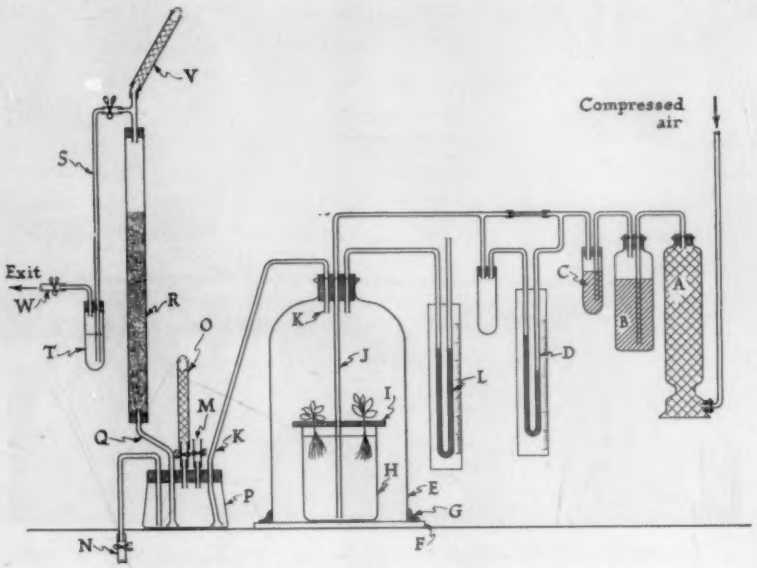


FIG. 2. Diagram of one unit of the apparatus.

cury valves and traps. A general view of the apparatus is shown in figure 3.

The rate of flow of air through the apparatus was kept at 6 liters per hour, which experience proved was sufficient to provide very efficient scrubbing by the barium hydroxide solution in the bead tower. The indicator trap, T, always remained clear, showing that the air was completely freed from carbon dioxide in the tower. The pressure in the respiration chamber was maintained at 1 atmosphere, as indicated by the levels of the *n*-butyl phthalate in the attached manometer, L. Very slight adjustments of the 2 taps were required for this, and the apparatus was kept under continual observation during all experiments to maintain the required uniform conditions.

The procedure was briefly as follows: Before sealing down the bell jar, the plants, supported on the wooden disc (I) so that their roots dipped into the nutrient solution in the battery jar, H, were arranged so that the air entering by the tube, J, would bubble into the solution. The bell jar was sealed into position and tested for leaks by clamping off K,K and noting the movements of the liquid in the manometer, L. K,K was unclamped and the carbon-dioxide-free air was allowed to flow through the apparatus for one hour. This period had previously been established as more than sufficient to renew the air in the apparatus. Next, a measured amount of standard barium hydroxide and a drop of phenolphthalein were placed in the titra-

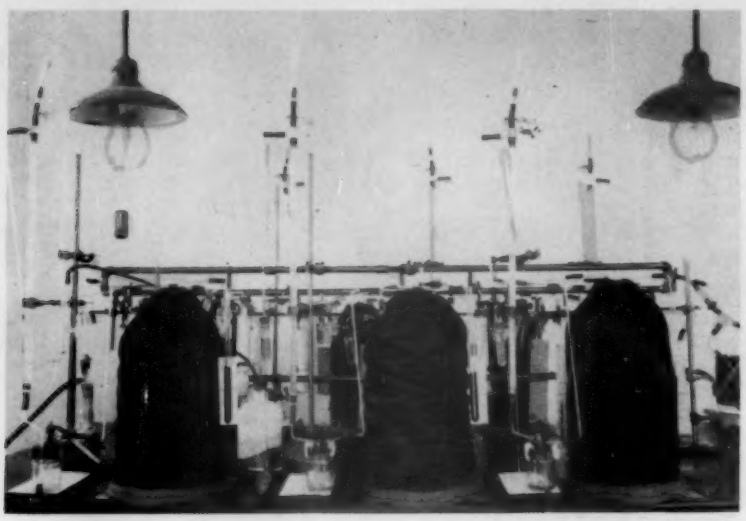


FIG. 3. General view of the respiration apparatus. (Photograph by J. A. CARLILE.)

tion flask through M, which was then shut by a pinch clamp. The two vents protected by soda lime tubes at O and V and the siphon N, previously filled with distilled water, were closed. The vacuum tap, W, was opened enough to draw the liquid in the flask up the glass tower and to maintain atmospheric pressure in the respiration chamber and a uniform rate of flow.

After a definite time interval—usually 4 hours—the vent O was opened, the compressed air and vacuum taps were shut, the vent V was opened, and the tube S was closed, in this order. The barium hydroxide solution was washed down from the tower with carbon-dioxide-free water through the opening made by removing V. The solution was titrated with standard hydrochloric acid solution through the opening M. The burette, connected to an acid reservoir, was permanently attached to the apparatus and was moved to each unit as required. The flask was emptied and rinsed with

carbon-dioxide-free water by means of the siphon N, which was left filled with water and clamped off during the experiment.

Results

The respiration rates were all calculated in terms of milligrams of carbon dioxide evolved per gram of fresh weight per hour. The results can best be understood by reference to the curves in figure 4, which are typical examples taken from each age group. It will be seen that the respiration rates of the healthy and diseased plants began at approximately equal levels and remained equal, within experimental error, up to the point at which the respiration rate of the diseased plants rose suddenly above the rate of the healthy plants. In the course of a few days the higher rate of the diseased plants gradually decreased and approached the rate of the healthy plants.

The special significance of these results lies in the observation that the sudden rise in the respiration rate of the inoculated plants coincided with the time at which the virus became systemic, *i.e.*, at the time of the clearing of the veins. Usually when the mosaic mottling appeared on the plant, the respiration rates of the diseased and the healthy plants were either almost or actually equal again. In most cases the experiments were not continued past that point. When the disease became systemic the diseased plants showed a negligible increase of weight whereas the output of carbon dioxide remained the same or was much greater than formerly so that the resulting respiration rate was much higher.

If the conditions were such that the clearing did not show, the rise in respiration rate occurred at such a period before the mottling that it was reasonable to assume that the systemic spread of the virus had coincided with the rise, especially as other indications such as crinkling of the leaves were usually present. On one occasion, when circumstances prevented the observation of the clearing of the veins, the highest rise of respiration rate was missed, but the initial rise and subsequent fall were measured. On another occasion, one unit of the healthy plants showed a sudden rise in respiration: it was found that one of the plants was contaminated with the virus. In the same batch of plants one of the inoculated plants gave a sudden rise on the second day of inoculation. Again one of these plants was seen to be contaminated and, upon removing it, the respiration rate of the unit fell back to normal, rising on the 8th day when the disease became systemic in the remaining plants.

The respiration rate of the healthy plants varied from about 0.30 mg. CO_2/gm./hour for the young plants to about 0.075 mg./gm./hour for the older plants with a tendency to decrease regularly with increasing age and weight. With the inoculated plants the course of the respiration rate was

the same for the plants of all the ages examined, except that the respiration rate was approximately 50 per cent. greater than that of the corresponding healthy plant when the disease became systemic.

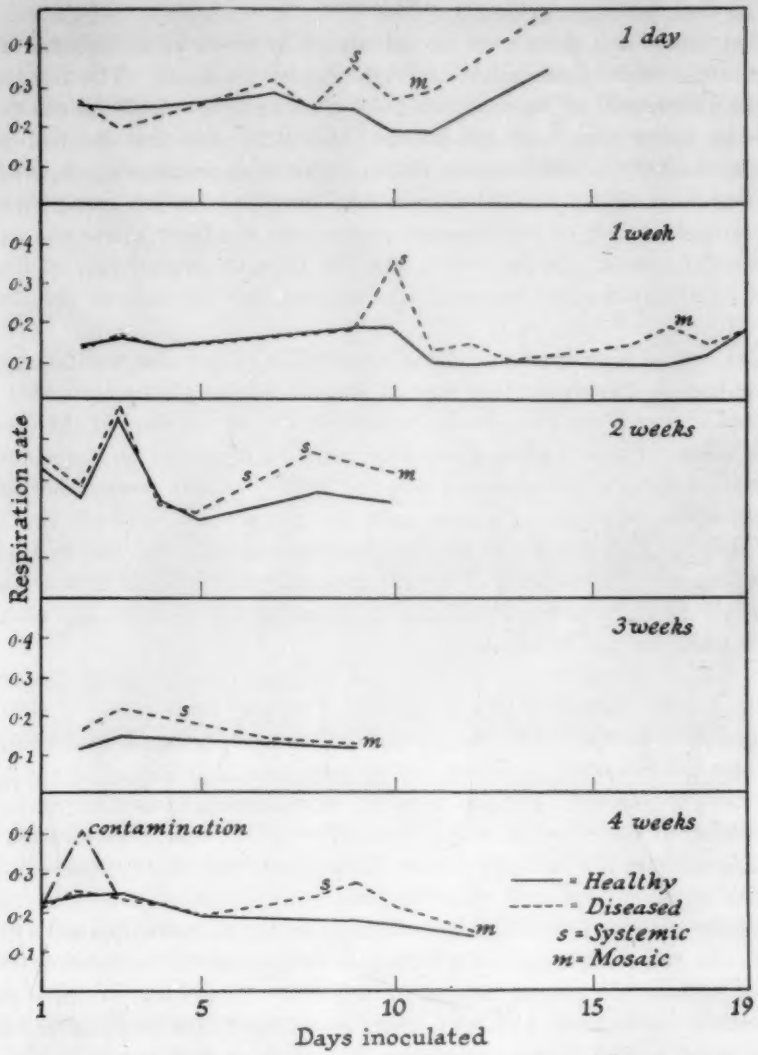


FIG. 4. Respiration rates, in milligrams CO_2 per gram of fresh weight per hour, of healthy and diseased plants at different ages of inoculation.

The behavior of the plant according to its age is illustrated in the curves of figure 5. The respiration curve of an average healthy plant is shown by a continuous line. The percentage increase in respiration rate of the dis-

eased plants inoculated at the various ages is drawn in a series of dotted curves. It will be seen that the percentage rise, when the disease becomes systemic, is approximately the same irrespective of the ages of the healthy plants at the time of inoculation. When the mosaic develops, the percentage rise apparently decreases with the increasing age of the inoculated plants.

These results indicate that at the time when the virus becomes systemic there is a great increase in the metabolic activity within the plant as indicated by the greater rate of respiration of the inoculated as compared with the healthy plant. A number of investigators have studied the changes and distribution of the virus subsequent to inoculation (**5, 7, 14, 16, 19**). Their results all indicate that with tobacco-mosaic virus the clearing-of-the-

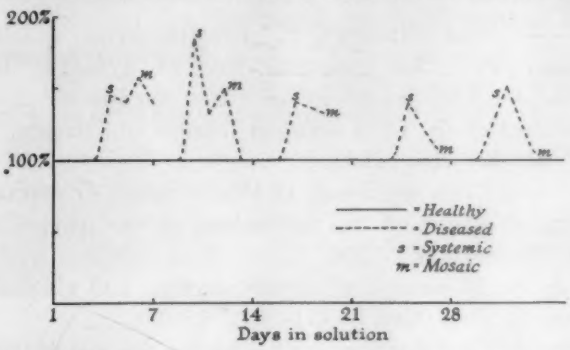


FIG. 5. The percentage increase in respiration rate of the diseased plants inoculated at various ages.

veins period corresponds to the rapid movement and increase of the virus, which would account for a high expenditure of energy whatever actual process is taking place. Further experiments are now being carried out on the respiration problems in order to obtain a fuller interpretation of these results.

Summary

The paper describes the apparatus and method for comparing the respiration of entire healthy and mosaic-diseased tobacco plants from the time of inoculation until the appearance of the mottling disease. The same plants were used throughout the period of each experiment.

It was found that the respiration ratio of the diseased plants and healthy plants remained at the same level until the disease became systemic. When the clearing of the veins was apparent, the respiration rate of the diseased plants rose rapidly, followed by a decrease until in the older plants it became approximately equal to that of the healthy plants by the time that the mosaic mottling had developed. The percentage increase in respiration rate

was approximately 50 per cent. higher than the rate of the corresponding healthy plants.

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
PRINCETON, NEW JERSEY

LITERATURE CITED

1. BENNETT, C. W. Relation of food translocation to movement of virus of tobacco mosaic. Jour. Agr. Res. **60**: 361–390. 1940.
2. BRONFENBRENNER, J. Further studies on so-called bacteriophage. Proc. Soc. Exp. Biol. Med. **22**: 81–82. 1924.
3. ———. Does bacteriophage respire? Science n.s. **63**: 51–52. 1926.
4. ———, and REICHERT, P. Respiration of so-called filterable viruses. Proc. Soc. Exp. Biol. Med. **24**: 176–177. 1926–1927.
5. CALDWELL, J. The physiology of virus diseases in plants. V. The movement of the virus agent in tobacco and tomato. Ann. Appl. Biol. **21**: 191–205. 1934.
6. ———. The physiology of virus diseases in plants. VI. Some effects of mosaic on the metabolism of the tomato. Ann. Appl. Biol. **21**: 206–224. 1934.
7. CRAFTS, A. S. Movement of viruses, auxins, and chemical indicators in plants. Bot. Rev. **5**: 471–504. 1939.
8. DUFRÉNOY, J. Modifications pathologiques du métabolisme cellulaire chez les tabacs. Ann. Épiphyties **18**: 259–280, 281–316. 1932.
9. DUNLAP, A. A. The total nitrogen and carbohydrates, and the relative rates of respiration, in virus-infected plants. Amer. Jour. Bot. **17**: 348–357. 1930.
10. GLASSTONE, V. F. C. Passage of air through plants and its relation to measurement of respiration and assimilation. Amer. Jour. Bot. **29**: 1942. (In press.)
11. HOAGLAND, D. R., and ARNON, D. I. The water-culture method for growing plants without soil. Univ. California Agr. Exp. Sta. Circ. 347. 1939.
12. HOLMES, F. O. Inoculating methods in tobacco mosaic studies. Bot. Gaz. **87**: 56–63. 1929.
13. ———. Local and systemic increase of tobacco mosaic virus. Amer. Jour. Bot. **17**: 789–805. 1930.
14. ———. Movement of mosaic virus from primary lesions in *Nicotiana tabacum* L. Contrib. Boyce Thompson Inst. **4**: 297–322. 1932.
15. KEMPNER, W. Chemical nature of the oxygen-transferring ferment of respiration in plants. Plant Physiol. **11**: 605–613. 1936.

16. KUNKEL, L. O. Movement of tobacco-mosaic virus in tomato plants. Phytopath. **29**: 684–700. 1939.
17. LEMMON, P. Comparative studies on metabolism of healthy and mosaic-infected tobacco leaves. Respiration studies. (Abstract) Amer. Jour. Bot. **22**: 912. 1935.
18. MACK, W. B. The relation of temperature and the partial pressure of oxygen to respiration and growth in germinating wheat. Plant Physiol. **5**: 1–68. 1930.
19. MATSUMOTO, T. Serological studies on the distribution and concentration of tobacco mosaic virus in host plants. I, II. Trans. Nat. Hist. Soc. Formosa **31**: 201–215, 275–285. 1941.
20. THUNG, T. H. Physiologisch onderzoek met betrekking tot het virus der bladrolziekte van der aardappelplant, Solanum tuberosum L. Tijdschr. Plantenz. **34**: 1–74. 1928.
21. WHITEHEAD, T. The physiology of potato leaf-roll. 1. On the respiration of healthy and leaf-roll infected potatoes. Ann. Appl. Biol. **21**: 48–77. 1934.

GROWTH OF *RHIZOPUS SUINUS* AS AFFECTED BY CERTAIN GROWTH FACTORS

CLAIR L. WORLEY

Introduction

A relatively large amount of work has been done relevant to definite compounds which show growth-promoting activities. A review of the investigations dealing with the effects of these known growth factors for lower plants, exclusive of yeast, reveals several interesting aspects. The degree of the necessity of any factor referred to is limited by the conditions of the test conducted and by the strain of the organism used. Although many authors list certain substances as being essential for certain species, the possibility that said compound can be substituted for by another compound is not obviated. It is of interest to note that the bios-complex (including thiamin and its fractions) was the first discovered and that it has been shown to be of primary importance not only for yeasts, but also for many other fungi, bacteria, certain animals, and even non-chlorophyllous parts of higher plants.

The inclusion of mesoinosite as a growth factor might be considered as fallacious. Mesoinosite has proved to be essential to none of the test organisms used; it is inert to the majority; and in those cases where it induces increase in growth, it is used in great enough concentrations to be classified as a nutrient. Biotin, on the other hand, does accelerate growth in certain organisms even when supplied in very minute quantities. This latter compound has been shown to be especially effective in conjunction with alanine and other growth factors. KÖGL and TÖNNIS (**4**) report that biotin is the most potent growth factor known, in certain cases, being effective in concentrations of one part in 400 million.

Thiamin (anuerin), or vitamin B_1, has proved to be the most universally effective, not only with respect to the components of the bios complex, but also to all known growth factors yet tested on the lower plants. In the majority of cases, this compound has proved to be either essential or stimulatory for the growth of the test organisms. In several instances it exerts no effect, and in other cases it is reported as being inhibiting. Thiamin is a complex molecule composed of two ring nuclei each of which may exist as an individual compound; *viz.*, as pyrimidine and thiazole. A study of this compound and of its components reveals an interesting diversity among those fungi for which the vitamin is effective as an essential growth factor. In this respect, fungi may be divided into the following five categories: (a) Those which require the *intact* thiamin molecule. A substitution of the two components is non-effective; (b) that group which requires both com-

ponents but is not so exacting as to require the intact vitamin compound; (c) those microorganisms which require pyrimidine alone; (d) those microorganisms which require thiazole alone; (e) those species which develop on synthetic media devoid of external supplies of thiamin or of its components.

In addition to these and other known chemical compounds, which have been tested as accessory growth factors, much work has appeared relative to the effectiveness of extracts and of concentrates of the tissues and products of various organisms. This field, however, is too large and too diverse to review in a specific manner.

The following series of experiments were conducted to investigate not only certain chemical compounds but also some unknown chemical complexes in relation to growth promoting properties as displayed through the effects on *Rhizopus suinus* Niel. Since this fungus develops quite well on purely synthetic media, it undoubtedly is capable of synthesizing all of its essential growth factors. Any substance which, when added in minute traces, results in a significant growth increase may be interpreted as being a stimulator. On the other hand, it is also possible that the fungus may synthesize an essential factor at a sub-maximal rate and that a growth increase may result from an external supply of this essential growth factor.

The techniques, species, chemicals, and assays used in the subsequent tests are the same, unless stated otherwise, as those employed in a previous investigation (**7**). All the data presented in the subsequent tables represent several replicas; enough to assure a biological significance of the results. The factor F is the mathematical mean on the nine original values and the value SE is its standard error.

Experimentation

Test I

The effect of yeast extracts on the gowth of *Rhizopus suinus*

Extracts, fractionations, and concentrates of yeast have proved important sources of vitamins and of growth factors for microorganisms. It therefore seemed advisable to test such products on this fungus.

A total of 7.70 gm. of "Red Star" baker's yeast was extracted for 15 minutes, at steaming temperatures (90° C.), with 150 ml. of distilled water. The solid matter was removed by centrifuging and discarded. The liquid was decanted, brought up to 200 ml., and tested in full strength and in dilutions of 1:10 and 1:100. An aliquot of 100 ml. of the above supernatant liquid was treated at room temperatures (25° C.) for a period of 30 minutes with 10 gm. of Nuchar–W. The charcoal was removed by centrifuging and the supernatant liquid was brought to mark and tested in the dilutions given above.

An alcoholic extract was made from another sample of the same yeast. A sample weighing 9.92 gm. was extracted 20 minutes in 150 ml. of hot 95 per cent. ethanol and the residue removed by centrifugal force. The supernatant liquid was reduced to 5 ml. by evaporation. To this was added 50 ml. of water, the solution being then boiled to remove the remaining traces of the ethanol. This latter solution, after cooling, was brought to 200 ml. and tested in the dilutions given above.

The cultural technique follows. A 25-ml. portion of the Rhizopus nutrient solution (7) and 5 ml. of the test solution were placed in 125-ml. Erlenmeyer flasks, stoppered with cotton wadding, and autoclaved at 12 pounds pressure for a period of 20 minutes. After cooling, they were inoculated with 1 ml. of the standard spore suspension of *Rhizopus suinus*, and then incubated at 25 ± 0.5° C., in diffused light, for 5 days. At the close of the culture period the mats were removed by filtration, dried in an oven, and weighed.

The prepared solutions were:

Cont.	Control.
Alc Y–100	Alcoholic yeast extract (0.01 conc.).
Alc Y–10	" " " (0.10 conc.).
Alc Y	" " " (1.00 conc.).
TY–100	Charcoal treated aqueous yeast extract (0.01 conc.).
TY–10	" " " " " (0.10 conc.).
TY	" " " " " (1.00 conc.).
UY–100	Untreated aqueous yeast extract (0.01 conc.).
UY–10	" " " " (0.10 conc.).
UY	" " " " (1.00 conc.).

TABLE I

THE EFFECT OF YEAST EXTRACTS ON THE GROWTH OF *Rhizopus suinus*

CULTURE	RHIZOPUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE	SPORULATION
	mg.		
Cont.	43.9	1.00	++
Alc Y–100	35.4	0.81 ± 0.013	++
Alc Y–10	25.5	0.59 ± 0.014	++
Alc Y	25.6	0.59 ± 0.013	++
TY–100	87.1	1.99 ± 0.034	+++
TY–10	46.0	1.05 ± 0.018	++
TY	46.5	1.05 ± 0.024	++
UY–100	26.2	0.60 ± 0.021	+
UY–10	38.1	0.88 ± 0.018	++
UY	66.3	1.51 ± 0.023	+++

The results with yeast extracts were diverse and contradictory to those obtained by other workers dealing with different microorganisms. Treat-

ment with 95 per cent. ethanol removed a material toxic to vegetative growth, but this treatment showed no effect on sporulation. An untreated aqueous extract was inhibiting in low concentration, but accelerated growth when used in higher concentrations. An aqueous extract treated with Nuchar–W, on the other hand, was stimulative throughout, althought varying greatly with the concentration used. The effects on sporulation showed a parallelism with the effects on dry weight yield. No attempt is made to explain these limited and varying results.

Test II

The effects of liver extract and of human urine on the growth of *Rhizopus suinus*

Animal tissues and animal products have been ready sources of growth factors. Fresh veal liver and male human urine, therefore, were chosen as representatives of this group of sources. Fresh veal liver was ground finely in a food chopper, and 250 gm. were suspended in 100 ml. of cold distilled water. This mixture was brought to the boiling point, the flame removed, and the preparation permitted to stand 15 minutes. This was filtered and the residue retreated in like manner with 50-ml. quantities of water until a total of 500 ml. of filtrate was obtained. The combined filtrates were reheated to boiling to coagulate the proteins and other colloids, these being removed by filtration. The new filtrate was placed at 8° C. for 4 hours. During this cold treatment, a considerable quantity of flocculent material appeared and this was filtered off while cold. The final filtrate was permitted to reach room temperature, was brought to mark, and finally tested at the dilutions listed below. One sample of urine was tested in the untreated state. The second sample was cleared with 10 per cent. Nuchar–W prior to testing.

The following solutions were prepared and tested:

Cont.	Control.
UU	Untreated urine.
TU	Charcoal treated urine.
L–5000	Liver extract (where 5 ml. soln. = 0.001 gm. liver).
L–500	" " (" " " = 0.010 gm. ").
L–50	" " (" " " = 0.100 gm. ").
L–5	" " (" " " = 1.000 gm. ").

The stimulating effects of the two higher concentrations of liver extract, as reported in table II, may be partially explained as the result of added energy-producing material. The results obtained with the two lesser concentrations of the liver extract and with the two samples of urine, however, are undoubtedly due to the presence of unknown growth factors. Other tests have shown that traces of zinc, the most effective of trace elements

TABLE II

THE GROWTH OF *Rhizopus suinus* AS AFFECTED BY URINE AND BY VEAL LIVER EXTRACTS

CULTURE	RHIZOPUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE	SPORULATION
	mg.		
Cont.	8.2	1.00	+
UU	42.3	5.16 ± 0.369	++−
TU	37.4	4.51 ± 0.095	++
L–5000	11.9	1.46 ± 0.039	+
L–500	14.6	1.79 ± 0.068	+−
L–50	27.2	3.33 ± 0.086	++
L–5	92.2	11.29 ± 0.226	++++

on this strain, augments vegetative growth to a far less degree than that obtained above. It is, therefore, tentatively assumed that all of the results just discussed are primarily manifestations of added growth factors.

TEST III

THE EFFECT OF SUBSTANCE-B ON THE GROWTH OF *Rhizopus suinus*

The Rhizopus filtrates were prepared under standard conditions given in a preceding paper (7). The test technique was that of test I above; the concentrations alone being varied as shown in table III.

TABLE III

THE GROWTH OF *Rhizopus suinus* AS AFFECTED BY ADDITIONS OF FILTRATES (SUBSTANCE–B) FROM OTHER CULTURES OF THE SAME FUNGUS

NUTR: FILTRATE: H_2O	RHIZOPUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE	SPORULATION
ml.	*mg.*		
25: 0:5	47.3	1.00	+
25: 5:0	63.0	1.33 ± 0.005	++
20:10:0	65.4	1.38 ± 0.010	++
15:15:0	71.3	1.51 ± 0.020	+++
10:20:0	74.0	1.56 ± 0.006	++++
5:25:0	70.9	1.49 ± 0.006	+++++

The results included in table III clearly indicate the presence of a growth factor for *Rhizopus suinus*. Since no new elements were introduced, minor elements can play no direct part in the explanation of these results. The explanation is probably to be found in the formation of a new or of a modified substance (substance–B ?) by a metabolic process or processes. Once again there is a near parallelism between the effectiveness of the sporulation factor and that of the growth factor. The results for the last solution is the only one to deviate from this generality.

TEST IV

THE INFLUENCE OF BIOS COMPONENTS ON GROWTH OF *Rhizopus suinus*

The bios complex and its components have proved to be important growth factors for a great number of related and unrelated microorganisms. The more important reports dealing with fungi include those of KÖGL and FRIES (3) on various fungi, of FRIES (2) on wood destroying and other fungi, of LINDEBERG (5) on species of Marasmius, and of MELIN and LINDEBERG (6) on mycorrhizal fungi. Similar tests were conducted on *Rhizopus suinus* to ascertain whether it responded to these substances in the same manner. The chemicals used were:

Thiamin ..... Merck, cryst. no. 0.69271
Mesoinosite ..... Pfanstiehl no. 1883
Biotin ..... A 10 per cent. concentrate prepared and kindly supplied by Dr. P. M. WEST of the Univ. of Wisconsin

TABLE IV

THE EFFECT OF THIAMIN ON *Rhizopus suinus* GROWTH

THIAMIN PER FLASK	RHIZOPUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE	SPORULATION
γ	*mg.*		
0.0	17.3	1.00	++
0.01	23.9	1.37 ± 0.022	++
0.10	22.7	1.31 ± 0.015	++
1.00	21.8	1.25 ± 0.017	++

Thiamin accelerates dry weight production by this fungus. Amounts of 0.01 gamma per 30 ml. of total nutrient solution must exceed the optimal concentration, since an increase in thiamin content results in a diminution of total dry matter.

TABLE V

THE EFFECT OF MESOINOSITE ON THE GROWTH OF *Rhizopus suinus*

MESOINOSITE PER FLASK	RHIZOPUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE	SPORULATION
mg.			
0	17.3	1.00	++
1	20.5	1.19 ± 0.022	++
5	24.8	1.43 ± 0.019	++
10	27.1	1.56 ± 0.033	++

Rhizopus suinus, unlike most fungi, but similar to certain strains of yeast, is stimulated markedly by the addition of mesoinosite to the medium. There exists, however, the possibility that this substance functions as a nutrient rather than as a growth factor.

TABLE VI

EFFECT OF BIOTIN ON DRY WEIGHT YIELD OF *Rhizopus suinus*

BIOTIN PER FLASK	RHIZOPUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE	SPORULATION
γ	*mg.*		
0.0	14.1	1.00	+
0.001	13.2	0.93 ± 0.044	+
0.010	16.1	1.14 ± 0.043	+
0.100	10.6	0.75 ± 0.013	+

Although the results obtained are contradictory, there is some evidence that biotin exerts an inhibitory effect on the vegetative growth of this fungus. The only other fungus for which a similar result has been reported is *Tricholoma pessundatum* (**6**).

TEST V

EFFECT OF COMBINATIONS OF THIAMIN, BIOTIN, AND MESOINOSITE ON THE GROWTH OF *Rhizopus suinus*

Other work on different species of fungi and on yeast has shown that these components may function in one manner when used in conjunction with another component and in another when used singly. Combinations, similar to those used by others, were tried with Rhizopus.

TABLE VII

GROWTH OF *Rhizopus suinus* AS AFFECTED BY COMBINATIONS OF THIAMIN AND MESOINOSITE

THIAMIN PER FLASK	MESOINOSITE PER FLASK	RHIZOPUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE	SPORULATION
γ	*mg.*	*mg.*		
0.0	0	17.3	1.00	++
1.00	10	27.0	1.56 ± 0.029	++
1.00	5	27.1	1.56 ± 0.021	++
1.00	1	30.1	1.74 ± 0.022	++
0.10	10	26.3	1.51 ± 0.035	++
0.10	5	22.0	1.28 ± 0.016	++
0.10	1	28.4	1.64 ± 0.046	++
0.01	10	24.1	1.40 ± 0.020	++
0.01	5	27.4	1.58 ± 0.014	++
0.01	1	21.5	1.24 ± 0.022	++

Thiamin and mesoinosite separately and combinations of thiamin and mesoinosite function as stimulating growth factors for *Rhizopus suinus,* but they fail to exert any influence on the extent of fruiting. These results are

in accordance with expectations based on work of others. When employing the combined growth factors the yields are less than the sums of the increases obtained for the two substances separately. Additions of 1 gamma thiamin + 1 mg. mesoinosite and of 0.1 gamma thiamin + 1 mg. mesoinosite proved to be the more efficient combinations.

TABLE VIII

GROWTH OF *Rhizopus suinus* AS AFFECTED BY COMBINATIONS OF THIAMIN AND BIOTIN

THIAMIN PER FLASK	BIOTIN PER FLASK	RHIZOPUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE	SPORULATION
γ	γ	*mg.*		
0.0	0.0	14.1	1.00	+
1.00	0.01	16.3	1.15 ± 0.026	++
0.10	0.10	15.8	1.11 ± 0.015	+−
0.10	0.01	16.8	1.19 ± 0.051	+−
0.01	0.01	15.9	1.12 ± 0.023	++
0.001	0.001	17.1	1.22 ± 0.032	+

The results of the tests reported above showed that thiamin stimulated, and biotin inhibited, vegetative growth of this fungus. These same effects are shown when these two factors are supplied concurrently. The data of table VIII, however, show that the effect of the added thiamin, in all cases, is greater than the opposite effect of the added biotin, and as a consequence all combinations tested were positively effective. Even though the last test solution contained the lowest thiamin content per flask, it possessed the greatest effectiveness. This may result from the very small inhibiting effect exerted by the low concentration of the biotin.

TABLE IX

GROWTH OF *Rhizopus suinus* AS AFFECTED BY COMBINATIONS OF MESOINOSITE AND BIOTIN

MESOINOSITE PER FLASK	BIOTIN PER FLASK	RHIZOPUS: AVERAGE DRY WEIGHT OF MYCELIUM	F ± SE	SPORULATION
mg.	γ	*mg.*		
0.0	0.0	14.1	1.00	+
10.0	0.01	22.2	1.57 ± 0.028	++
5.0	0.10	36.9	2.61 ± 0.067	+−
5.0	0.01	23.1	1.63 ± 0.028	++
1.0	0.01	75.6	5.34 ± 0.205	++++
0.5	0.001	19.6	1.39 ± 0.025	+−

In no case did the biotin manifest an inhibition equal to or greater than the stimulation exerted by the mesoinosite. All combinations, therefore,

resulted in an increase in dry weight. The extent of this increase for the combination of 1 mg. mesoinosite + 0.01 gamma biotin was unexpected and unaccounted for, although similar results were obtained when it was repeated two other times. Otherwise the results are as might be predicted from the effects exerted by the two substances individually.

Test VI

Vegetative growth of *Rhizopus suinus* as affected by synthetic bios complexes

A combination of various quantities of crystalline thiamin, mesoinosite, and biotin is the nearest approach to a synthetic bios complex known. Since bios proves to serve as a growth factor for many lower plants and since naturally occurring bios exerts diverse effects on *Rhizopus suinus* (*cf.* test I), synthetic bios complexes were tested on this species. Several combinations were used since the proportions of the components vary with the strain of yeast from which they are isolated.

TABLE X

Growth of *Rhizopus suinus* as affected by synthetic bios complexes

Thiamin per flask	Meso-inosite per flask	Biotin per flask	*Rhizopus*: average dry weight of mycelium	F ± SE	Sporulation
γ	*mg.*	γ	*mg.*		
0.0	0.0	0.0	14.1	1.00	+
0.10	10.0	0.01	32.7	2.31 ± 0.043	++
0.10	5.0	0.10	17.3	1.21 ± 0.038	++
0.10	5.0	0.01	30.4	1.92 ± 0.101	++
0.10	1.0	0.10	32.3	2.28 ± 0.060	++
0.10	1.0	0.01	21.6	1.52 ± 0.056	+++
0.01	10.0	0.01	21.7	1.54 ± 0.024	++
0.01	5.0	0.01	21.7	1.53 ± 0.029	+-
0.01	1.0	0.01	24.4	1.73 ± 0.036	+-
0.001	0.5	0.001	19.8	1.40 ± 0.026	+-

All readily available types of synthetic bios functioned as stimulating growth factors for growth (and sporulation) for *Rhizopus suinus*. The more effective proportions of thiamin/mesoinosite/biotin were: 0.1/10,000/0.01 and 0.1/1000/0.01; while the least effective was 0.1/5000/0.1. A survey of the data included in table X shows that the ultimate results are manifestations of the balance of the stimulating effects of thiamin and mesoinosite on the one hand and of the inhibitory effects of biotin on the other.

Although the above results are not identical with those which have been obtained for other species, they are similar, and it may be tentatively stated that *Rhizopus suinus* is affected by bios and its components in a manner

quite similar to *Tricholoma pessundatum* (**6**); *Marasmius foetidus, M. androsaceus,* and *M. perforans* (**5**); *Nematospora Gossypii* (**1, 3**); *Nectria coccinea, Sclerotinia cinerea,* and *Polyporus adustus* (**3**).

Discussion and conclusions

Yeast, the natural source of bios, has been used most frequently as an organism from which growth factors may be isolated. Almost without exception, aqueous and alcoholic extracts of yeast have stimulated the vegetative and reproductive development of fungi and of bacteria. The effects of yeast extracts on *Rhizopus suinus,* however, have been diverse and contradictory. An untreated aqueous extract of a Baker's yeast exerted either inhibiting or stimulating effects, depending upon the concentration employed, while the same extract cleared with 10 per cent. Nuchar–W was stimulating throughout all concentrations tried. The yeast fraction soluble in 95 per cent. ethanol, on the other hand, was slightly toxic to vegetative growth but without effect on sporulation. In general, these preliminary tests show that yeast itself (or the accompanying portions of the cake) contains water soluble stimulators and inhibitors, the latter being removable by adsorption on Nuchar–W or by solution in 95 per cent. ethanol. It is impossible to ascertain from the limited data whether the inhibiting factor adsorbed on the charcoal and the one dissolved by the alcohol are the same or different. Are they unknown toxins, or are they members of the heteroauxin group which occur in yeast and which are deleterious to the normal development of *Rhizopus suinus*? There also exists the possibility that a component of the bios complex, or the complex itself, might hinder the growth of the fungus.

Both thiamin and mesoinosite stimulated vegetative growth regardless of the manner of application or of the concentration used. Biotin, on the other hand, exerted a significant inhibition. The response of Rhizopus to biotin was not in accord with those obtained with other species which have been investigated. In all cases, however, the positive effects of thiamin and of mesoinosite were greater than the negative effect of the biotin (for the concentrations used), so that in combination of either or of both of the former with biotin there was increased growth. The results tend to suggest the possibility that the stimulant present in the yeast extract may be thiamin or mesoinosite, or both, and that the inhibitor is not a component of bios but rather an unknown, or possibly heteroauxin. A parallelism existed between the effects on growth and on asexual reproduction for all tests involving yeast extracts and synthetic bios components. It is thus indicated that these factors affect the fruiting of *Rhizopus suinus* only indirectly through altered vegetative growth.

Liver infusion and urine were tested as animal sources of growth factors.

Both gave marked positive results when assayed by *Rhizopus suinus*. These results are in agreement with those obtained on other species of fungi. Although trace elements might also play a rôle here, the procedure followed eliminated the possibility of added nutrients.

Rhizopus filtrate (substance–B) was likewise tested as a representative of a fungal source of growth factors for fungi. This test clearly proved the presence of a biologically produced growth factor, which is not only effective on other fungus species, as shown in previous tests, but is also effective in respect to the growth and the asexual reproduction of *Rhizopus suinus* itself. All the tests, however, failed to show whether the growth factor was formed by altering the nature of a compound in the substrate or whether it was secreted by the living protoplasm. The possibility also remains that it was freed during autolysis of the older hyphae.

Sincere thanks and appreciation are hereby extended to Dr. B. M. Duggar, of the University of Wisconsin, under whom this work was conducted and the protocol of this paper prepared.

University of Georgia
Athens, Georgia

LITERATURE CITED

1. Fries, N. Untersuchungen über Bios-artigen Substanzen als Wachstumsfaktor für holzzerstörende Polyporaceen. Svensk. Bot. Tidskr. **31**: 42–46. 1937.
2. ————————. Über die Bedeutung von Wuchsstoffen für das Wachstum verschiedener Pilze. Symb. Bot. Upsalienses **3**(2): 1–188. 1938.
3. Kögl, F., and Fries, N. Über den Einfluss von Biotin, Aneurin und Meso-Inosit auf das Wachstum verschiedener Pilzarten. Zeit. physiol. Chem. **249**: 93–110. 1937.
4. ————————, and Tönnis, B. Über das Bios-Problem. Darstellung von krystallisiertem Biotin aus Eigelb. Zeit. physiol. Chem. **242**: 43–73. 1936.
5. Lindeberg, G. Über das Wuchsstoffbedürfnis verschiedener Arten Pilzgattung Marasmius. Svensk. Bot. Tidskr. **33**: 85–90. 1939.
6. Melin, E., and Lindeberg, G. Über den Einfluss von Aneurin und Biotin auf das Wachstum einiger Mykorrhizenpilzen. Bot. Not. **1939**: 241–245. 1939.
7. Worley, C. L. Agencies affecting the production of substance B by *Rhizopus suinus*. Plant Physiol. **16**: 461–480. 1941.

A STUDY OF THE CHEMICAL COMPOSITION OF SOYBEANS DURING MATURATION

ALVIN C. WOLFE, J. B. PARK, AND R. C. BURRELL

Introduction

During the course of some soybean breeding experiments at The Ohio State University, the questions arose as to what extent the high quality of a green edible variety could be correlated with chemical composition; and whether the stage of development at which the green beans are most palatable might be linked in any way with changes in chemical composition. A large amount of analytical work was undertaken in which the changes that occurred in the seeds of four varieties of soybeans were determined as they approached maturity. The present paper will be limited to reporting certain data that seem to have considerable interest from a physiological viewpoint.

Most of the previous detailed analyses of soybeans have been limited to the mature beans. STREET and BAILEY (**16**) in 1915 reported a rather comprehensive analysis of a Hollybrook variety of soybean. STARK (**15**), SHUTT (**13**), KHARICHKOV and VAGANOVA (**8**), SMIRNOVE and LAVROVA (**14**), and CARTTER and MILNER (**5**) have made studies of soybean composition with especial reference to conditions which affect the oil and protein content. SASAKI and TO (**12**) separated the embryo and seed coat and analyzed each portion. BURRELL and WOLFE (**3**) analyzed 5 varieties of mature soybeans including both vegetable and field varieties; and WOODRUFF and KLAAS (**18**) at the University of Illinois have carried out very extensive investigations of the composition of both green edible and field varieties. They found, among other things, that with maturing soybeans, protein increases irregularly, while fat and sugar contents increase regularly when the percentages are expressed on a moisture-free basis. They conclude that soybean sugars are converted into other constituents such as fats during the maturation of the beans.

Materials and methods

Samples were taken on different dates from the same plantings; and also, to minimize climatic effects, other samples were collected on the same date from several plantings of the same variety which had been made at different times in order to give different stages of development on the same sampling day. Bansei, Kura, Jogun and Illini were the varieties chosen.

The pods were quickly stripped from the plants, taken to the laboratory where they were blanched with a large quantity of boiling water, and quickly shelled by hand. Of each sample of beans, 500 grams were boiled for 15

minutes with sufficient 95 per cent. aldehyde-free ethyl alcohol (to which one gram of $CaCO_3$ was added to neutralize acidity) so that, taking into account the approximate amount of moisture in the beans, the resulting solution should have a concentration of very nearly 80 per cent. ethyl alcohol. A record was kept of the number of beans in each sample. The samples were preserved in sealed glass jars, more 80 per cent. alcohol being added if necessary to completely cover the beans.

For the moisture determination, two 10-gram samples of unblanched beans were dried in an oven at 110° C. The nearly dried beans were coarsely ground in a mortar to facilitate drying. Similar samples of blanched beans were treated in the same way, so that appropriate corrections could later be applied to compensate for the water that had been taken up by the blanched samples.

Ash was determined by charring the finely ground beans from the moisture determination over a low flame and ashing them overnight in an electric muffle furnace which was set at a temperature of 650° C.

Two 10-gram samples of unblanched beans were boiled in toluene for 10 minutes to destroy lipase activity. Tests confirmed no enzyme activity in samples which had been treated in this way. These samples were stored in tightly stoppered bottles and were used for fat and crude fiber determinations. For the fat determinations, the beans, already partially extracted by the toluene, were separated, dried, ground, placed in weighed Gooch crucibles which contained asbestos mats, and extracted in a Pickel apparatus with ether. Fat was calculated from the weight of the completely extracted residue and also from the fat recovered from the toluene and ether extracts. For crude fiber, the fat-free residues, still in the Gooch crucibles, were treated according to the standard method for the determination of crude fiber. Extraction of the 500-gram samples for the protein and carbohydrate analyses was accomplished in a specially designed extractor (**2**). Before carrying out the extraction, the preserved beans were ground to a coarse pulp. When extraction was complete, the alcoholic extract was allowed to stand overnight. The greenish oil which separated was removed, along with the excess $CaCO_3$, and weighed. The alcoholic washings were combined with the main solution and the whole diluted to a volume of 6 liters with alcohol. Nitrogen, sugar, solids of extract and similar determinations were carried out on aliquots of this extract. In general, "Official Methods" were used (**1**) or at least very similar methods which had been adapted to this type of material. Some further modifications did have to be made in certain instances on account of the high fat content of the alcoholic solution. For example, in the sugar determinations, 500-ml. aliquots were evaporated on the steam bath to approximately 100 ml. each. To the cold solution, 200 ml. of ethyl ether and 50 ml. of water were added, the mixture gently shaken

and allowed to stand overnight. Separation of the ether layer was effected by use of a separatory funnel. The ether layer was washed twice with water to insure complete recovery of the sugars. The water solution was then clarified with neutral lead acetate and the excess lead removed with sodium sulphate. The filtered solution was made up to 500 ml. and 50-ml. aliquots were used for reducing sugar and sucrose determinations.

Calcium, magnesium and phosphorus were determined in the ash by a method similar to that used by MORRIS, NELSON and PALMER (**10**) for the determination of these elements in cattle excreta and feeding stuffs.

Results of all these analyses are tabulated in tables I and II. In table I, the percentages of the various components at different stages of development are expressed on the moisture-free basis. Sucrose, raffinose, stachyose and any other di- or trisaccharides that might have been present and were hydrolyzable with invertase or hydrochloric acid were all determined together and are expressed as sucrose. Reducing sugars are expressed as glucose; as are also hemicelluloses. Table II gives the composition in terms of milligrams per average weight bean in each sample. Values in the tables are averages for two separate determinations.

Discussion

Based on observations such as those of DE LUCA (**6**) with the developing olive; LECLERC DU SABLON (**9**) with walnut and almond; RUSHKOVSKI (**11**) for sunflower seed; CASKEY and GALLUP (**4**) for cottonseed; and to a less extent on studies of respiratory quotients (**7**), the view has been put forward that in fatty seeds, the fats are formed chiefly at the expense of carbohydrates of the seeds which are transformed into fats during maturation. On the other hand, THOR and SMITH (**17**) found that in the pecan, the decrease in sugar content of the whole fruit during the period of oil synthesis would account for not more than 5 per cent. of the final amount of oil.

The data here presented for the soybean seem to indicate that much of the fat must be formed from materials that are brought into the seed from other parts of the plant at the time of synthesis. With reference to this interpretation, it will be seen that the analyses based merely on percentages of the various components in the whole dry weight samples may be confusing. Curves constructed from these percentage analyses generally show irregular increases or decreases. When, however, the same data are expressed in terms of milligrams of the various components per average bean, it is apparent that in almost all instances, there is a regular increase in the quantity of each component per bean during maturation. Even those components like starch, which on the percentage basis seem to be definitely decreasing as the beans mature, are found to be actually increasing when expressed in terms of milligrams per bean. This anomaly arises, of course,

TABLE I

DATES OF PLANTING, DATES OF HARVEST, MOISTURE CONTENT AND PERCENTAGE COMPOSITION OF FOUR VARIETIES OF SOYBEANS (MOISTURE-FREE BASIS) AT DIFFERENT STAGES OF DEVELOPMENT

VARIETY	DATE OF PLANTING	DATE OF HARVEST	MOISTURE (FRESH WEIGHT) BASIS	ASH	NITROGEN* OF RESIDUE	NITROGEN OF EXTRACT	CRUDE FAT	CRUDE FIBER	SUCROSE	REDUCING SUGARS	DEXTRINS	STARCH	HEMICELLULOSES	PENTOSANS	Ca	Mg	P
			%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Bansei	6– 4–38	8–22–38	71.36	5.59	5.16	0.77	14.45	5.96	6.42	3.84	0.52	1.49	9.44	4.82	0.329	0.389	0.586
"	"	8–29–38	73.70	5.51	5.03	0.44	18.39	5.12	8.92	2.72	0.80	2.59	8.53	3.90	0.274	0.294	0.603
"	"	9– 3–38	67.23	5.29	5.31	0.41	19.13	4.08	9.24	2.12	0.76	2.61	8.76	3.87	0.224	0.263	0.674
"	"	9– 9–38	67.67	5.49	5.59	0.26	20.57	4.32	6.18	1.51	1.17	2.55	8.01	4.25	0.229	0.296	0.707
"	"	9–15–38	69.61	5.56	5.49	0.44	20.25	4.76	8.26	1.66	1.22	3.06	9.05	4.14	0.293	0.305	0.749
"	"	9–21–38	66.45	5.49	5.81	0.31	19.30	4.62	7.18	1.61	1.44	1.91	8.33	4.42	0.302	0.300	0.717
Illini	6– 4–38	9– 3–38	71.64	5.08	5.13	0.27	18.17	5.97	6.81	2.98	1.21	3.56	8.46	4.04	0.269	0.223	0.648
"	"	9– 9–38	65.93	5.38	5.11	0.21	20.66	4.99	5.92	2.27	1.57	3.37	7.22	4.37	0.256	0.234	0.681
"	"	9–15–38	67.50	5.21	5.38	0.175	20.96	5.07	4.84	1.84	1.13	2.15	7.84	4.41	0.238	0.259	0.703
"	"	9–21–38	63.17	5.39	5.62	0.23	21.07	4.73	6.63	1.85	0.84	2.19	7.02	4.19	0.263	0.265	0.787
Kura	6– 4–38	8–31–38	77.46	5.47	5.24	0.63	15.45	5.56	7.09	0.53	0.63	7.11	8.07	4.24	0.226	0.225	0.618
"	"	9– 5–38	73.55	5.11	5.20	0.51	17.99	5.03	8.87	1.07	1.09	4.68	7.02	3.79	0.239	0.230	0.598
"	"	9–10–38	68.22	5.08	5.92	0.47	18.10	4.84	8.70	0.86	0.97	4.18	8.58	4.09	0.213	0.238	0.655
"	"	9–16–38	66.49	5.31	6.06	0.31	19.75	4.61	6.41	0.87	0.75	4.39	7.51	4.19	0.255	0.244	0.703
"	"	9–22–38	65.34	5.46	6.05	0.29	20.65	4.24	7.42	0.90	0.87	4.51	6.67	4.18	0.308	0.261	0.737
"	"	9–28–38	63.89	5.46	6.17	0.38	18.99	4.60	8.41	0.61	1.07	2.24	8.31	3.90	0.283	0.248	0.733
Jogun	4–23–38	8–27–38	81.74	5.38	4.89	1.10	14.32	5.34	9.79	2.31	1.12	5.11	5.60	4.50	0.195	0.224	0.564
"	5– 3–38	"	79.89	5.80	5.17	0.79	18.12	5.35	5.65	1.75	1.17	6.08	6.28	4.41	0.242	0.239	0.533
"	5–17–38	"	74.66	5.13	5.07	0.64	17.69	4.59	10.33	1.33	0.61	6.00	5.52	4.18	0.222	0.210	0.513
"	6– 1–38	"	70.86	5.17	5.40	0.52	19.29	4.32	9.65	0.88	1.32	5.13	5.34	3.91	0.224	0.224	0.512
"	6– 9–38	"	70.52	5.62	5.40	0.45	20.89	4.12	9.40	1.30	0.89	4.17	5.67	3.95	0.236	0.236	0.597

* Nitrogen may be converted to protein by multiplying by the factor 5.70.

TABLE II

COMPOSITION OF FOUR VARIETIES OF SOYBEANS AT DIFFERENT STAGES OF DEVELOPMENT, EXPRESSED AS MILLIGRAMS OF EACH COMPONENT PER AVERAGE BEAN

Variety	Stage of development*	Average weight per bean	Moisture	Ash	Nitrogen of residue	Nitrogen of extract	Crude fat	Crude fiber	Sucrose	Reducing sugars	Dextrins	Starch	Hemicelluloses	Pentosans	Ca	Mg	P
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Bansei	I	109.9	78.4	1.8	1.63	0.24	4.6	1.9	2.0	1.2	0.2	0.5	3.0	1.5	0.10	0.12	0.18
"	II	294.1	216.8	4.3	3.89	0.34	14.2	4.0	6.9	2.1	0.6	2.0	6.6	3.0	0.21	0.23	0.47
"	III	434.8	292.3	7.5	7.57	0.58	27.3	5.8	13.2	3.0	1.1	3.7	12.5	5.5	0.32	0.38	0.96
"	IV	526.3	356.1	9.3	9.51	0.44	35.0	7.4	10.5	2.6	2.0	4.3	13.6	7.2	0.39	0.50	1.20
"	V	588.2	409.4	9.9	9.82	0.79	36.2	8.5	14.8	3.0	2.2	5.5	16.2	7.4	0.52	0.55	1.34
"	VI	588.2	390.9	10.8	11.46	0.61	38.1	9.1	14.2	3.2	2.8	3.8	16.4	8.7	0.60	0.59	1.41
Illini	I	147.0	105.3	2.1	2.14	0.11	7.6	2.5	2.8	1.2	0.5	1.5	3.5	1.7	0.11	0.09	0.27
"	II	222.2	146.5	4.1	3.87	0.16	15.6	3.8	4.5	1.7	1.2	2.4	5.5	3.3	0.17	0.18	0.52
"	III	294.1	198.5	5.0	5.19	0.17	20.0	4.85	4.6	1.8	1.1	2.4	7.5	4.2	0.23	0.25	0.67
"	IV	322.6	203.8	6.4	6.68	0.27	25.4	5.6	7.9	2.2	1.0	2.3	8.3	5.0	0.31	0.31	0.94
Kura	I	256.4	198.6	3.2	3.03	0.36	8.9	3.2	4.1	0.3	0.4	4.1	4.7	2.5	0.13	0.13	0.36
"	II	370.4	272.4	5.0	5.10	0.59	17.6	4.9	8.7	1.1	1.1	4.6	6.9	3.7	0.23	0.23	0.59
"	III	555.6	379.0	9.0	10.45	0.83	32.0	8.6	15.4	1.5	1.7	7.4	15.2	7.2	0.38	0.42	1.16
"	IV	625.0	415.6	11.1	12.69	0.65	41.4	9.7	13.4	1.8	1.6	9.2	15.7	8.8	0.53	0.51	1.47
"	V	690.0	450.8	13.1	14.47	0.69	49.4	10.1	17.7	2.2	2.1	10.8	16.0	10.0	0.74	0.62	1.76
"	VI	769.2	491.4	15.2	17.14	1.06	52.8	12.8	23.4	1.7	3.0	6.2	23.1	10.8	0.79	0.69	2.04
Jogun	I	303.0	247.7	3.0	2.70	0.61	7.9	3.0	5.4	1.3	0.6	2.8	3.1	2.5	0.11	0.12	0.31
"	II	384.6	307.3	4.5	4.00	0.61	12.9	4.1	4.4	1.4	0.9	4.7	4.9	3.4	0.19	0.19	0.41
"	III	555.6	414.8	7.2	7.14	0.90	24.9	6.5	14.5	1.9	0.9	8.5	7.8	5.9	0.31	0.30	0.72
"	IV	606.1	429.5	9.1	9.54	0.92	34.1	7.6	17.0	1.6	2.3	9.1	9.4	6.9	0.43	0.40	0.90
"	V	625.0	440.8	10.4	9.95	0.83	38.5	7.6	17.3	2.4	1.6	7.7	10.4	7.3	0.46	0.44	1.10

* For corresponding dates of planting and of harvest, see table I.

because other components of the bean are increasing in weight so rapidly that the relative, but not the total quantities of those substances which are present in much smaller amounts, do actually decrease.

Another interesting observation that can be made from these data has to do with the considerable quantity of undetermined substances which must be present. These amount to 13 to 20 per cent. of the dry weight even after all the nitrogen is converted to a corresponding protein value. STREET and BAILEY (**16**) were able to account for all but 8.60 per cent. of the dry weight of mature soybeans by a similar analysis.

Summary and conclusions

An analysis of four varieties of soybeans at different stages of development indicates that fat and protein of the seed are probably produced largely from substances brought into the seed from other parts of the plant at the time of synthesis, rather than from the carbohydrates already present in the seed.

Special attention is called to the desirability of expressing certain data in terms of quantity per plant or per plant part (as per average seed) rather than only in terms of the percentages present in the whole sample.

Various modifications of the usual methods of analysis have been employed to render them more suitable for use with this type of fresh, high-fat plant material.

DEPARTMENT OF AGRICULTURAL CHEMISTRY AND
DEPARTMENT OF AGRONOMY
THE OHIO STATE UNIVERSITY

LITERATURE CITED

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis. Third edition. Washington, D. C. 1930.
2. BURRELL, R. C., and WOLFE, ALVIN C. A convenient extractor for use with larger quantities of plant material. Jour. Chem. Ed. **18**: 513. 1941.
3. ————, and WOLFE, ALVIN C. A comparative study of the chemical composition of five varieties of soybeans. Food Res. **5**: 109–113. 1940.
4. CASKEY, C., and GALLUP, W. D. Changes in the sugar, oil and gossypol content of the developing cotton boll. Jour. Agr. Res. **42**: 671–673. 1931.
5. CARTTER, J. L., and MILNER, R. T. Proc. 17th Ann. Meeting Amer. Soybean Assoc. Pp. 12–15. 1937.
6. DE LUCA, S. Recherches sur la formation de la matière grasse dans les olives. Compt. Rend. Acad. Sci. (Paris) **53**: 380–384. 1861. Ibid. **55**: 470–473; 506–510. 1862.

7. GERBER, C. Recherches sur la formation des reserves oleagineuses des graines et des fruits. Compt. Rend. Acad. Sci. (Paris) **125**: 732–735. 1897.
8. KHARICHKOV, V., and VAGANOVA, E. Chemical composition of soybeans under the conditions prevailing in the middle Volga district. Masloboino Zhirovoye Delo 1930, no. 2, 64–66.
9. LECLERC DU SABLON, M. Sur la formation des reserves non azotées de la noix et de l'amande. Compt. Rend. Acad. Sci. (Paris) **123**: 1084–1086. 1896.
10. MORRIS, H. P., NELSON, J. W., and PALMER, L. S. Quantitative determination of calcium, magnesium and phosphorus in feedstuffs and cattle excreta. Ind. Eng. Chem. Anal. Ed. **3**: 164–167. 1931.
11. RUSHKOVSKII, S. Changes in the chemical composition of sunflower seeds with the sowing time. Masloboino Zhirovoye Delo 1930, no. 9–10, 43–56.
12. SASAKI, S., and TO, S. Cell wall constituents of soybeans. Chemical constituents of the seed coat. Jour. Agr. Chem. Soc. Japan **15**: 624–628. 1939. Also: Bull. Agr. Chem. Soc. Japan **15**: 105–106. 1939.
13. SHUTT, FRANK T. Influence of heredity and environment on the protein and oil content of soybeans. Canadian Dept. Agr. Rept. Dominion Chemist. 1930: 54–57. 1931.
14. SMIRNOVA, M. I., and LAVROVA, M. N. Variability in the chemical composition of different varieties of soybeans. Bull. Appl. Bot. Gen., Plant Breed. (U.S.S.R.) Ser. 3, no. 5, 73–102. 1934.
15. STARK, R. W. Environmental factors affecting the protein and oil content of soybeans and the iodine number of soybean oil. Jour. Amer. Soc. Agron. **16**: 636–646. 1924.
16. STREET, J. P., and BAILEY, E. M. The carbohydrates and the enzyme of the soybean. Ind. Eng. Chem. **7**: 853–8. 1915.
17. THOR, C. J. B., and SMITH, C. L. A physiological study of the seasonal changes in the composition of the pecan during fruit development. Jour. Agr. Res. **50**: 97–122. 1935.
18. WOODRUFF, S., and KLAAS, H. A study of soybean varieties with reference to their use as food. Univ. Ill. Agr. Exp. Sta. Bull. 443. 1938.

APPLICATION OF THE LIQUID EXTRACTION METHOD FOR THE DETERMINATION OF TOTAL ORGANIC ACIDS IN PLANT SAP[1]

T. L. Isaacs and T. C. Broyer

(WITH ONE FIGURE)

Introduction

The method of Pucher, Vickery, and Wakeman (**6**) for the estimation of total organic acids by titration in tobacco leaf tissue has proved useful in studies of organic acid metabolism. The method described by Pucher and co-workers was devised for the extraction of organic acids from dried plant tissues. The present paper describes the application of the liquid extraction method for the titrimetric estimation of total organic acids in expressed plant sap with a discussion of the advantages offered by, and problems arising from, such a procedure. The method is based on the observation that the organic acids can be directly extracted by ether in an efficient liquid extraction apparatus, provided the sap is previously acidified with sulphuric acid (**10**).

Description of apparatus

A sketch of the apparatus is shown in figure 1. The continuous ether liquid extractor is designed from a Soxhlet extractor as described by Vickery and Pucher (**10**). The apparatus is fitted with ground glass joints to make the ether extraction more efficient. The condenser (B, fig. 1) is built with a double condenser unit to economize on solvent etc., as described in a recent publication by Yip (**13**). A separate vial (C, fig. 1) having a height[2] suitable for the aliquot desired is placed into the Soxhlet (A, fig. 1). A funnel (D, fig. 1) having small apertures at the bottom is placed in position inside the vial. The extraction flask (E, fig. 1) contains 100 ml. of H_2O_2-free ether. Heat is supplied by a constant temperature water bath maintained at a temperature of 50° C. It is desirable that the temperature be sufficiently high to adequately resupply solvent (after condensation) for rapid, continued extraction. The condensed ether dropping into the funnel causes a pressure head to be developed. The solvent is forced through the small openings of the funnel at the bottom of the vial removing the organic acids as it passes through the liquid to be extracted. The stream of ether globules produces enough agitation of the liquid to insure stirring at all

[1] Clerical assistance in the preparation of this publication was furnished by the personnel of Works Project Administration Official Project no. 65–1–08–91–B–10.

[2] It is important that the height of the ether layer be relatively short so that the extracting solvent may be rapidly replaced by the incoming flow of fresh ether. The rate of partition-extraction is thus enhanced.

times. Extraction is continued for a period of 20 to 24 hours to insure completeness. For plant tissues containing organic acids slightly soluble in ether, the time limit may have to be extended (see table I, sample 11).

Method and results

Barley plants were grown according to the method of HOAGLAND and BROYER (4). Sap was expressed from frozen excised barley roots by the

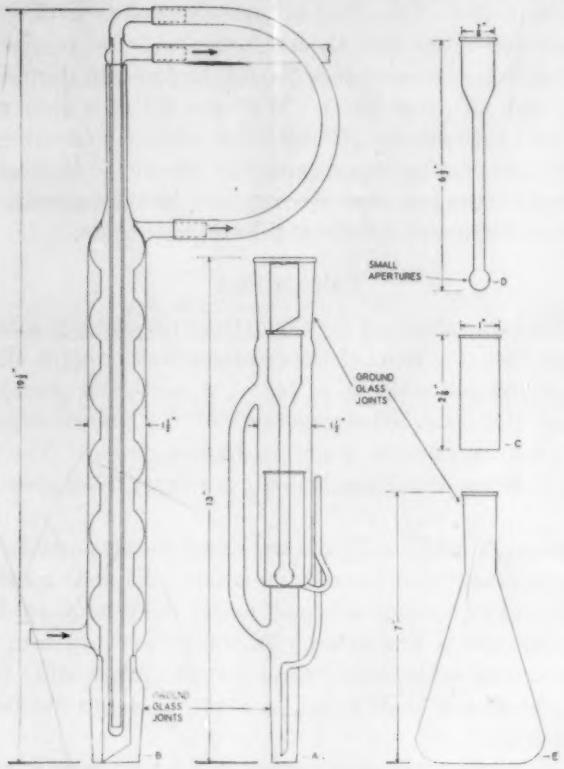


FIG. 1. Sketch of apparatus for liquid extraction of ether soluble organic acids. A. Soxhlet extractor with standard ground glass joints. B. Double consender unit with standard ground glass joint. C. Glass vial. D. Funnel unit consisting of a funnel and attached Folin bulb. E. Receiving flask with standard ground glass joint.

experimental technique of BROYER and HOAGLAND (1). As has been pointed out in another paper (2), certain methods of pretreatment are considered as unsatisfactory in organic acid estimations. Although in the present study barley root saps were employed, the method may also be applied to plant tissues.

A suitable aliquot of fluid, 10 to 15 ml., is acidified to pH 1 with sulphuric

acid[3] and placed in the liquid extraction vial within the Soxhlet. The funnel is inserted, receiving flask attached, and the extraction started. A blank is conducted with distilled water, parallel with the organic acid sample, being refluxed for the same period of time, and subsequently carried through the same series of operations.

After the extraction is completed, 25 ml. of CO_2-free water is added to the receiving flask. The contents are gently agitated to distribute the acids into the aqueous phase. The ether is carefully distilled off under reduced pressure at approximately 50° C. and condensed for re-utilization. The aqueous solution is transferred to a 100-ml. beaker and diluted to approximately 50 ml. with CO_2-free water. With the aid of a glass electrode, the extract is titrated between the pH limits 7.8 and 2.6, according to the principle of the method of VAN SLYKE and PALMER (**9**). If sulphuric acid is used in the titration the resultant mixture may be subsequently used for the individual determination of the component organic acids.

Calculations

The titration value obtained for the extract necessitates a correction factor owing to the fact that none of the common barley root acids are titrated to the extent of 100 per cent. (**6**, p. 141). A correction factor of 1.05 was calculated from the dissociation constants of the known organic acids in barley root sap between the two given pH limits selected. The total acidity[4] was expressed in terms of milliequivalents per gram fresh weight of original plant tissue.

In the presence of oxalic acid it is necessary to carry out an independent determination of oxalic acid in order to correct the final result (**11**), since oxalic acid behaves as a monobasic acid under the conditions described. A similar correction would be necessary in the presence of large amounts of phosphoric or tartaric acids; and to a lesser extent nitric acid. (The amounts of oxalic and phosphoric acids found in barley root sap did not necessitate a correction factor.)

For certain experimental treatments organic acid factors might have to be estimated for each individual treatment. This would be necessary if and when the individual acids involved are differentially modified (**3**). Under such conditions, because of the laborious procedures involved in separating and estimating individual organic acids, a method not involving acid corrections might prove more suitable. Such a method has been proposed by

[3] A highly ionized mineral acid is employed, since in the final titration traces of these acids will be present as the salts of strong electrolytes and will not alter the titration value for total organic acids between pH 7.8 and pH 2.6 (**9**, p. 570).

Sulphuric acid is selected rather than hydrochloric acid since the latter has been shown experimentally to convert glucose to organic acid on heating (**12**, p. 808).

[4] Any carbonic acid in the original sample is completely expelled during the extraction.

ULRICH (8). As pointed out elsewhere, however, such a procedure may be subject to other, more serious limitations. Under the experimental conditions so far studied, the individual acids have been modified in similar manner, so that a correction factor established for the control material may be satisfactorily applied to the experimental treatments. It may be noted that the procedure of direct titration of ether-soluble non-volatile organic acids (8) would include the sum of the equivalents of sulphate, phosphate, and nitrate present in the material.

In the material tested, ether-soluble organic bases occurred in extremely small amounts and did not significantly influence the results. If other plant

TABLE I

TOTAL ORGANIC ACIDS IN EXPRESSED SAP OF EXCISED BARLEY ROOTS

SAMPLE NO.	ORGANIC ACIDS IN MILLIGRAM EQUIVALENTS × 10^2 PER GRAM OF FRESH TISSUE	
	LIQUID EXTRACTION METHOD	BUFFER TITRATION CURVES pH 7.8 TO 2.6
	mg. eq. × 10^2	*mg. eq.* × 10^2
1	3.44 3.30 3.32	3.60
2	3.76 4.10 4.13	4.02
3	3.37 3.30	3.29
4	4.75 4.77	4.56
5	3.40 3.40	3.62
6	2.40 2.38	2.09
7	3.71 3.80	3.04
8	4.18 4.19	4.22
9	4.00 4.00	4.34
10	4.43 4.60 4.77	4.50
11	6.11* 6.10 6.09	6.36

* Extraction time, 56 hours. An average value of 4.3 was obtained by the normal 24-hour treatment.

tissues are studied a minor modification of the correction factor may be necessary.

In order to show the applicability of the method, these organic acid estimates were compared with results obtained on comparable samples of sap by calculating total organic acids from buffer titration curves between the pH limits of 7.8 and 2.6. The results of the two independent determinations are presented in table I. Replicate determinations by the liquid extraction method were generally satisfactory. Favorable agreement was obtained between the two methods compared. These facts indicate that this liquid extraction procedure yields a valid quantitative estimate of the total organic acids present in the sample.

Discussion

PUCHER and VICKERY (**7**) have described a mechanically operated continuous liquid extraction apparatus. This equipment affords certain advantages such as reduced fire hazard, direct extraction of large volumes of fluids, and use of solvents (ethyl acetate) more efficient than ether in extracting organic acids. The extract obtained, however, is not applicable for subsequent acid titration (**7**, p. 657). The method herein applied incorporates essentially all of the features (aside from fire hazard, which can be minimized in other ways (see fig. 1)) offered by the Widwark apparatus, as modified by PUCHER and VICKERY. The present technique presents no difficulty with respect to titration errors arising from solvent decomposition.

It should be pointed out that all methods so far proposed for extraction and titration of total organic acids under various metabolic treatments, entail certain assumptions. The direct titration of sap, obtained from tissues after adequate freezing and pressing, equal to the sum of the individual titers between the pH of the original sap and about pH 2.5 and pH 8.0, respectively, yields an acid equivalent value which will serve at least as an approximation of the total organic acids. Under reasonably controlled conditions such values may serve adequately to interpret certain generalized metabolic changes (**5**). In order to estimate more carefully the total organic acids, a separation and estimation of the individual acids, in order to apply a satisfactory correction on the total, is necessary. The technique herein used allows for the application of these various procedures on an individual aliquot of unknown material.

The liquid extraction method for the determination of total organic acids has several advantages not offered by methods involving dehydration (**6**, **8**, **11**) of plant tissues or fluids. The organic acids are extracted directly and immediately, thus insuring maximum stability of these compounds. This may prove valuable in studies of organic acid metabolism involving unstable intermediates, in that there is no time lag for the activity of microorganisms to become important. The direct extraction minimizes any possible loss of

volatile organic acids. The design of the apparatus permits the use of a wide range of aliquots, within semi-micro quantities. The use of a vial prevents siphoning over of impurities, including fixed acids, encountered with thimble extraction. This method, besides being theoretically sound, is quicker than others which require a lengthy preparation of the sample. As previously indicated, the method may be applied to fluid from other types of tissues with a possible modification of the correction factor. It is suggested that fresh tissues may be directly used, since ether will denature proteins and destroy the permeability of the cells, thereby causing a release of organic acids.

Summary

1. The liquid extraction method for the determination of organic acids is reviewed and a design of the apparatus given.

2. The method has been successfully applied to excised barley root sap.

The authors desire to express their appreciation to Mr. F. G. VIETS for analytical assistance. We are indebted to Dr. A. ULRICH for his suggestions during the preparation of this paper.

THE UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

LITERATURE CITED

1. BROYER, T. C., and HOAGLAND, D. R. Methods of sap expression from plant tissues with special reference to studies on salt accumulation by excised barley roots. Amer. Jour. Bot. **27**: 501–511. 1940.
2. ————, ISAACS, T. L., and VIETS, F. G. Effects of pretreatment on the estimation of organic acids in plant tissues. Plant Physiol. **17**: 69–79. 1942.
3. CLARK, H. E. Effect of ammonium and of nitrate nitrogen on the composition of the tomato plant. Plant Physiol. **11**: 5–24. 1936.
4. HOAGLAND, D. R., and BROYER, T. C. General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol. **11**: 471–507. 1936.
5. ————, and ————. Hydrogen-ion effects and the accumulation of salt by barley roots as influenced by metabolism. Amer. Jour. Bot. **27**: 173–185. 1940.
6. PUCHER, G. W., VICKERY, H. B., and WAKEMAN, A. J. Determination of the acids of plant tissue. II. Total organic acids of tobacco leaf. Ind. Eng. Chem. Anal. Ed. **6**: 140–143. 1934.
7. ————, and ————. A mechanically operated continuous liquid-extraction apparatus. Ind. Eng. Chem. Anal. Ed. **11**: 656–657. 1939.

8. Ulrich, A. Accumulation of mineral elements in relation to organic acid metabolism with special reference to root cells. Thesis, University of California, Berkeley, 1939.
9. Van Slyke, D. D., and Palmer, W. W. Studies of acidosis. XVI. The titration of organic acids in urine. Jour. Biol. Chem. **41**: 567–585. 1920.
10. Vickery, H. B., and Pucher, G. W. Chemical investigations of the tobacco plant. I. A preliminary study of the non-volatile organic acids of tobacco leaves. Connecticut Agr. Exp. Sta. Bull. 323: 155–202. 1931.
11. ————, and ————. Chemical investigations of the tobacco plant. IV. The effect of the curing process on the organic acids of tobacco leaves. Connecticut Agr. Exp. Sta. Bull. 352: 649–685. 1933.
12. ————, ————, Wakeman, A. J., and Leavenworth, C. S. Chemical investigations of the tobacco plant. VI. Chemical changes that occur in the leaves during culture in light and in darkness. Connecticut Agr. Exp. Sta. Bull. 399: 757–832. 1937.
13. Yip, J. S. Extraction and determination of Pyrethrin I in ground Pyrethrum flowers. An improved apparatus. Ind. Eng. Chem. Anal. Ed. **13**: 107–108. 1941.

THE NECESSITY OF ZINC FOR *PINUS RADIATA*

M. E. Smith and N. S. Bayliss

(WITH THREE FIGURES)

Introduction

In Western Australia a number of softwood plantations have been established on poor soils. Of the many species of Pinus planted, only *P. radiata* and *P. pinaster* are practicable on many of the areas. Even in these hardy species numerous disorders arise, fundamentally owing to soil poverty. Soil analyses (**5**) show that phosphate is extraordinarily low in the majority of cases. At the request of the Forests Department of Western Australia, the nutrition of *Pinus radiata* in culture solution was studied. Field experiments (**4, 5**) had shown that zinc deficiency, and probably phosphate deficiency, were the most common causes of disease. Zinc spraying, and fertilizing with superphosphate, have been resorted to on a large scale. A number of puzzling features related to "rosetting" (as the zinc deficiency disease is called) require an explanation, such as the very slow response to even large applications of a zinc salt to the soil, and the favorable results often obtained with superphosphate. There is reason to believe that the soil contains sufficient zinc for the needs of the plant, but that it is in some way rendered unavailable. The unavailability seems to be accentuated by clearing and burning of the native forest or scrub. It is conceivable that burning, followed by heavy rains, will result in almost complete loss of zinc from the surface layers (**3**) of a deep sandy soil such as the one in question. The problem is further complicated by the mycorrhizal habit of the pine. One would expect the associated fungal mycelium to be a more capable assimilator of zinc than the roots of non-mycorrhizal pines. And in fact analyses of fructifications of the mycorrhizal fungus indicate a much higher concentration of zinc than in healthy leaves of *Pinus radiata*. This suggests the possibility that in a zinc deficient soil, the fungus exerts a priority over the limited amount of zinc absorbed by the mycorrhizae.

Field trials (**5**) with diseased trees, using a large number of metals (including zinc, copper, manganese, boron, molybdenum, cobalt, iron, and magnesium) applied by injection, or by spraying the foliage with dilute solutions, showed that zinc alone remedied the "rosetting." This indicated that the disease is due to a deficiency of zinc, a conclusion which is supported by analyses in which the needles of "rosetted," "cured," and healthy untreated plants, exhibited a fairly definite increasing gradation of zinc content. It is not yet known, however, whether "rosetting" will subsequently recur in trees which have been "cured" by spraying, and the spray-

ing of large trees would not be practicable on a plantation scale. It is, therefore, of some interest to discover the characteristic symptoms of zinc deficiency *per se*, uncomplicated by a possible phosphate deficiency, and if possible, symptoms which will identify the disorder at an early stage in the field, so that timely corrective measures can be applied.

Zinc is now accepted as essential for the growth of fungi, also for the angiosperms in general, but to our knowledge, it has not been previously shown that zinc is necessary for the conifers. Since the anatomy of the coniferales is distinctly different from that of the angiosperms, it is not unreasonable to expect differences in the nutritional requirements. Indeed, the present studies have indicated that *Pinus radiata* is capable of growing in extraordinarily low concentrations of phosphate, and according to field observations (**5**), *Pinus pinaster* is even more remarkable in this respect.

Experimental technique

It was felt that contamination with dust might be an important factor in experiments with seedlings of *Pinus radiata*, which might conceivably grow for six months or more before exhibiting symptoms of zinc deficiency, on account of the low growth rate and the comparatively large seeds. For this reason, considerable care was exercised at all stages of the investigation to eliminate dust, and the apparatus was designed with this end in view. A large volume of air passes through the aerators in the culture solutions in the course of several months, and so it is of some importance to completely free this air from suspended matter. The air was supplied by a water-operated blast-pump (since a compressor was not available) in conjunction with a pressure regulator, and an arrangement of traps and cotton filters to remove spray and dust. To this system was connected a series of culture vessels, a satisfactory type of which is shown in figure 1. The green house was made as nearly dustproof as possible, and woodwork, metalwork, etc., coated with clear Duco or with Dulux, which do not contain appreciable amounts of zinc.

Pinus radiata seeds were collected from a single tree growing in a locality where "rosetting" is known to be of frequent occurrence, with the dual object of reducing the hereditary variation and limiting the reserves of zinc available to the seedling. The seeds were germinated on filter paper or on cheese cloth, in a covered Pyrex vessel. In 1939, trials were made to determine the most suitable culture solution for *Pinus radiata*. Of the many widely different solutions tested, that of ARNON (**1**) proved the most satisfactory. This was adopted for all subsequent work, with the modification that the only "microelements" included were boron, manganese, and copper (it was not necessary to add molybdenum because the purification did not remove this metal). The control solutions contained 0.5 p.p.m. of zinc, which, although slightly inhibitory at first, permitted very good growth after the seedlings had become accustomed to the high concentration.

For the elimination of zinc (and also of copper) from the stock solutions, a method[1] based upon extraction of the concentrated solutions with diphenylthiocarbazone proved to be convenient and very effective. It has as its primary objective the removal of zinc, but since most of the salts employed

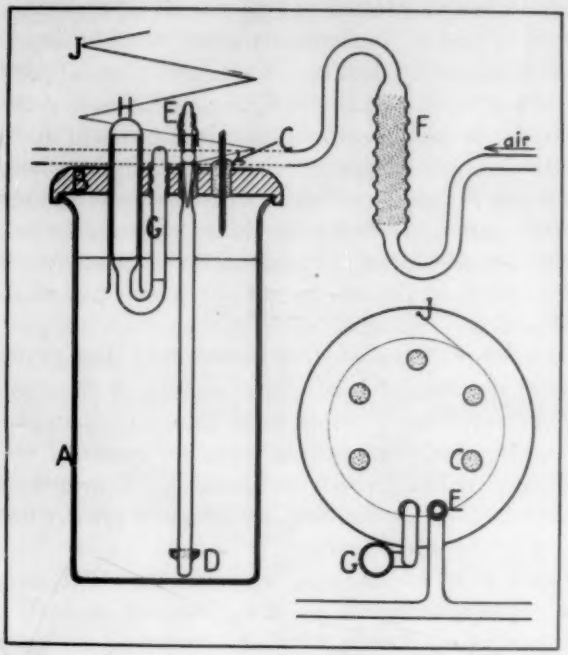


FIG. 1. Diagram of apparatus for growing *Pinus radiata* in culture solution.

A. Pyrex beaker, one liter capacity, covered with black paper and an outer layer of white paper.

B. Hardwood (jarrah) flanged cover, coated with clear duco.

C. Five plants supported with cotton wool in holes in the cover.

D. Sintered Pyrex glass aerator.

E. Glass needle-valve to adjust the rate of bubbling.

F. Filter of cotton wool.

G. S-tube through which water is added to offset transpiration losses, etc. (The level of solution in A can be ascertained without lifting B).

H. Inverted test-tube protecting the opening in G.

J. Aluminum wire spiral supporting the seedlings.

(B.D.H Analar, except the Baker's C.P. analyzed $Ca(NO_3)_2 \cdot 4H_2O$) contained considerable quantities of lead, the procedure was designed to remove this metal also. If the extractions are performed with thorough shaking, copper is removed fairly completely. Copper-free solutions were also prepared in this way, for later experiments.

[1] A similar method, involving preliminary co-precipitation of the heavy metals as phosphates, has been used by PIPER (6).

In the case of calcium nitrate, potassium nitrate, and magnesium sulphate, a concentrated solution of each salt is adjusted to a pH of about 8 by the addition of a few drops of aqueous ammonia. The solution is thoroughly extracted with a chloroform solution of dithizone.[2] A little H_2SO_4 is added to the separated aqueous solution to obtain a pH of approximately 5, and a final extraction is made. Immediately after purification, the solution is washed free of dithizone by shaking with successive small portions of chloroform. Chloroform remaining in the aqueous solution is removed by evaporating the mixture to dryness at a temperature of about 100° C., in a large silica basin, over which is suspended an inverted, slightly larger silica basin to exclude dust, etc. Finally, sufficient zinc-free water is added to the basin to make a molar solution, which is poured into a storage bottle. A somewhat different method is adopted for the purification of ammonium phosphate. A concentrated solution of ammonium monohydrogen phosphate is extracted at a pH of about 7.7. Sufficient pure sulphuric acid is added to reduce the pH to 5.5. Henceforth the procedure follows that already described.

In the case of the microelements, some samples of these salts were found to contribute to the culture solutions more impurities than the purified solutions of the major elements. It was possible, however, to select certain samples which required no further purification. Before the technique can be further refined, it will be necessary to achieve a greater degree of purity in the solutions of the microelements.

The total amount of the dithizone metals in the water, culture solutions, reagents, etc., was determined by the dithizone method, essentially as described by STOUT and ARNON (7). All water used contained less than 0.1 microgram of dithizone metals per liter. It was prepared by two distillations in a special all-pyrex still. Chloroform sufficiently free from the metals in question was obtained by two distillations in Pyrex. The sulphuric acid (B.D.H. Analar) was also twice distilled in Pyrex. The ammonia solution (8N) was prepared by standing a Pyrex bottle, containing pure water, in a large vacuum desiccator containing concentrated ammonia solution. After about a week at reduced pressure, the strength of the solution was determined, and water added to bring the normality to approximately 8. It is very important that the dithizone be carefully purified. A strong solution in chloroform is shaken with dilute aqueous ammonia. The clear portion of the aqueous solution is shaken with chloroform and a little dilute acid. This procedure of passing the dithizone from ammonia solution to chloroform solution is repeated two or three times, and the final chloroform solution is used immediately after its preparation.

[2] It is important to use as small an excess of dithizone solution as possible, so that after extraction only a small amount of uncombined dithizone remains, because it was found that dithizone generally contains large amounts of impurities which are not readily extracted from the aqueous phase.

Throughout the investigation, all the glass vessels, tubing, etc., employed were of Pyrex glass, except the extraction funnels (Wood's Resistance Glass), and the measuring cylinders. These, however, were always carefully cleaned immediately before use. Glass bottles, tubing, etc., were cleaned successively with chromic acid, hot hydrochloric acid, distilled water, and finally steamed for several hours.

Results

The plants were arranged in the greenhouse so that uniform light and heat conditions prevailed for pairs of treatments. Moreover, the control plants made more lateral growth than the zinc-deficient seedlings. Therefore, the height of each individual may be taken as a conservative indication of the relative growth of the whole shoot. Table I illustrates the differen-

TABLE I

COMPARISON OF SETS OF CONTROL AND ZINC-DEFICIENT SEEDLINGS OF *Pinus radiata* OF INCREASING AGE

AGE	HEIGHT		DESCRIPTION OF MINUS ZINC PLANTS AS COMPARED WITH CONTROLS
	CONTROL	MINUS ZINC	
days	*in.*	*in.*	
52	.....	.....	Very small, and indistinguishable in size and color
77	4.6	4.1	No symptoms apart from the slightly lower average height
101	6.8	5.0	Flat topped; apical buds a darker green
108	7.5	5.9	Flat topped; older needles drooping; one plant bronzing, others dark green
116	7.8	5.6	Flat topped; apical needles dark green and spirally twisted
141	11.4	6.2	Flat topped; secondary needles dark green and very short
170	10.0*	7.4*	Flat bunched tops; secondary needles short and stiff; bronzing apical buds

* These plants had no forced aeration.

tiation of height (average of five plants in most cases) in a series of seedlings of increasing age, as observed on July 30th. It will be evident that visible effects of zinc deficiency can be obtained in three to four months, depending upon the light intensity and the rate of growth. (Under conditions of high light intensity, it is probable that symptoms would appear much earlier.)

The first deficiency symptom to be observed was merely a decreased growth rate, evidenced by a darker green color of the young apical needles as compared with those of the controls. (Some factor, probably light, was limiting for the more rapidly growing seedlings. In consequence, newly

formed and actively growing apical needles of both control and deficient plants were a light green, while slowly growing apical needles in plants which had commenced to demonstrate their zinc deficiency were dark green. Nevertheless, this dark green was an apparently normal color, the same color indeed as the older needles below.) Until this stage, there was no detectable

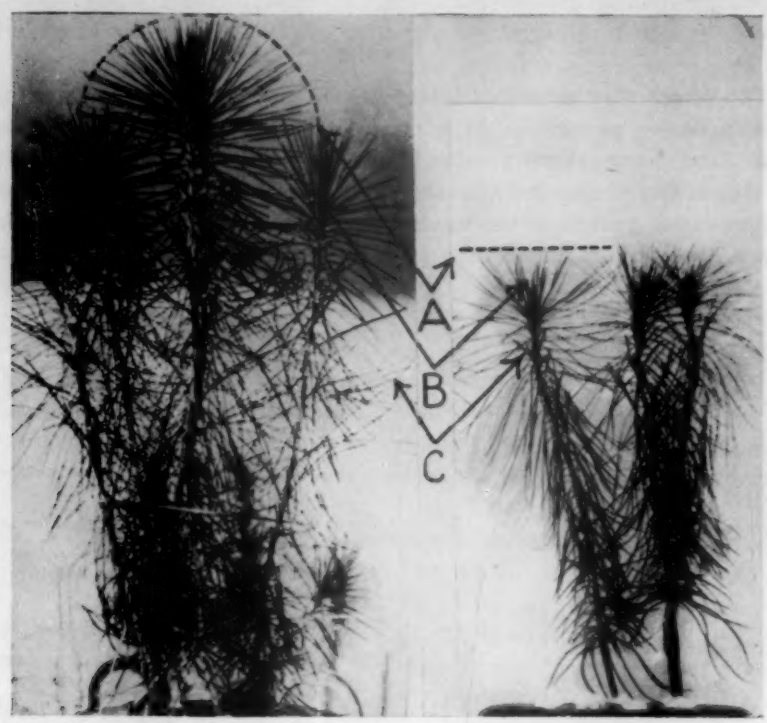


FIG. 2. Characteristics of early stages of zinc deficiency. Left, control; right, minus zinc.

A. Diagrammatic representation of the difference in outline of the apices. Note the flat topped appearance of the controls.

B. Immature apical needles folded inwards in zinc deficient plants, outwards in controls. (This can be seen more clearly when observed from a vertical position.)

C. Secondary needles in minus zinc seedlings very short and stiff, and fascicles generally unopened.

inferiority of the growth of zinc starved plants. A short time afterwards, however, the lower activity of their apical meristems resulted in a flat-topped appearance, which can be seen in figure 2. Apical buds of these plants assumed a conical, bunched formation, contrasting with the open centers of the controls. In some cases the zinc-deficient conical buds were spirally twisted, but this seemed to be a variable character. Secondary needles ceased growing, and were very short, thick, and stiff, and the fascicles did

not open (fig. 2). Notwithstanding, their color remained an apparently healthy dark green for several weeks. Generally, the secondary needles were approximately one-half inch long in both minus zinc and control plants when the first symptoms became evident. However, the secondary needles of the minus zinc plants never attained a length of one inch, while those of the controls attained a length of four or five inches within five weeks from their first appearance. It should be emphasized that the zinc-deficient plants retained their apparently healthy dark green color for approximately five weeks after they ceased growing. During this period there was no sign

FIG. 3. Comparison of root systems, showing apical swelling in zinc deficient plants. Left, control; right, minus zinc.

of injury whatsoever. Later, small yellow dots appeared on the needles near the apex. Browning followed, and soon the tops presented a bronzed appearance. Almost simultaneous with the first symptoms in the shoot, a characteristic conical swelling of the root tips (fig. 3) was observed in a number of plants. A similar swelling of the apical meristem has been observed in zinc-deficient tomato roots (**2**). It will be noticed in figure 3 that zinc-deficient plants possessed more short roots than the controls.

In order to throw some light upon the rôle of mycorrhizae in zinc deficiency of pines, it had been hoped to repeat the foregoing experiments with mycorrhizal plants. Attempts were not successful, however, in developing mycorrhizal roots on *Pinus radiata* in culture solutions, even after inocula-

tion with two of the fungal species commonly associated with pines in this country (*Rhizopogon luteolus* and an unidentified species of Boletus). The addition of glucose to the nutrient media, or the use of culture solutions which were unfavorable to the growth of the pine (*e.g.*, solutions containing a very low concentration of one or more of the necessary elements), were without avail in bringing about a mycorrhizal condition.

Summary

1. Nutrient solutions were purified with respect to zinc by extraction with a chloroform solution of diphenylthiocarbazone. The method is also applicable to the removal of copper.

2. Seedlings of *Pinus radiata* were grown in zinc-free culture solutions, and deficiency symptoms were obtained in three to four months. The plants were non-mycorrhizal.

3. Symptoms of zinc deficiency in *Pinus radiata* seedlings are slow growth rate (with consequent development of flat top); inwardly folded apical needles, which later exhibit a yellow mottling, followed by bronzing; and short, stiff, dark green secondary needles in unopened fascicles. The root tips may be distinctly swollen.

Sincere thanks are accorded to Mr. R. W. Pickering, of this department, and to Mr. S. L. Kessel, Conservator of Forests in Western Australia, for their advice and assistance. Grateful acknowledgment of financial assistance is made to the Hackett Fund of this University.

Department of Chemistry
University of Western Australia

LITERATURE CITED

1. Arnon, D. I. Microelements in culture-solution experiments with higher plants. Amer. Jour. Bot. **25**: 322–325. 1938.
2. Eltinge, E. T., and Reed, H. S. The effect of zinc deficiency upon the root of *Lycopersicum esculentum*. Amer. Jour. Bot. **27**: 331–335. 1940.
3. Goldschmidt, V. M. The principles of distribution of chemical elements in minerals and rocks. Jour. Chem. Soc. (London), Pt. 1, 655–673. 1937.
4. Kessel, S. L., and Stoate, T. N. Plant nutrients and pine growth. Australian Forestry **1**: 4–13. 1936.
5. ————, and ————. Pine nutrition. Western Australia Forests Department Bull. 50. 1938.
6. Piper, C. S. Molybdenum as an essential element for plant growth. Jour. Australian Inst. Agr. Sci. **6**: 162–164. 1940.
7. Stout, P. R., and Arnon, D. I. Experimental methods for the study of the rôle of copper, manganese, and zinc in the nutrition of higher plants. Amer. Jour. Bot. **26**: 144–149. 1939.

RESPONSE OF *CYPERUS ROTUNDUS* L. TO FIVE MOISTURE LEVELS

CHARLES HOMER DAVIS

Cyperus rotundus L. is a field weed known in all the Southern States as nut grass. The plant produces rhizomes, tubers, basal bulbs and fibrous roots below ground, and rosettes of leaves, scapes, and umbels above ground. The response of nut grass to moisture is an important factor in field eradication and studies were started in the greenhouse to determine the levels at which the plant showed differential response to soil moisture. The results reported herein constitute part of the data collected.

Pear, corn, and lemon plants have been shown to respond to differences in moisture well above the so-called wilting point by ALDRICH, WORK, and LEWIS (**1**), DAVIS (**2**), LOOMIS (**4**), and FURR and TAYLOR (**3**). The distance from the plant to moisture was also shown by ALDRICH *et al.*, DAVIS, and FURR and TAYLOR to be a factor in the availability of soil moisture for plant growth. In the experiments cited, growth was measured as the soil moisture was reduced, but because of mechanical difficulties the soil could not be maintained at any one moisture level.

Methods

The experiment was started August 12, 1940, with 45 squat-type gallon pots in which the rapid growth of the roots minimized the factors of distance. One tuber was planted in each pot. The fresh weight of the tubers varied from 1.01 to 2.24 gm. with an average weight of 1.43 gm. There was no significant difference in the weight of tubers planted, between the moisture series or between replications. After twenty-four weeks there was no correlation between the initial weight of tuber and final weight of tubers, either within a moisture series or over the entire experiment. There also was no significant correlation between the initial weight of tuber and either total or initial number of shoots.

The pots were divided into nine replications and so placed along the length of the greenhouse bench as to minimize variations in light intensity and temperature. The soil used to fill the pots was a heavy silt to which organic material and sand had been added a year before the pots were filled. The moisture equivalent of the mixture was 16.93 per cent. and the determined wilting percentage for corn was 8.22 per cent. The soil in the pots absorbed at least 22 per cent. of its oven dry weight of water when watered by standing in pans of water for 24 hours or until the surface was wet. The moisture differences were obtained by wetting uniformly after the pots had dried to different levels of moisture to simulate different periods between

wetting in the field. When the weight of a pot was within 45 gm. of its calculated low-moisture weight, or 1 per cent. of the 4.5 kgm. of soil contained, the pot was watered. Each pot was marked when daily weighings showed that it was approaching the low moisture point and was then checked twice each day or oftener when rapid evaporation was taking place.

One series of pots was watered when the gross weight of the pot showed a moisture percentage of 18; the second series was watered when the gross weight showed that a moisture content of 15 per cent. was approached; the third series was watered when the soil moisture was lowered to 12 per cent.; the fourth series was watered when the soil moisture was lowered to 9 per cent.; the fifth series was watered when the soil reached 6 per cent. of moisture. The moisture percentage contained in the pots was calculated at the time of filling and checked at the end of the experiment.

Sudden increases in the temperature and wind velocity in the open greenhouse increased the rate of evaporation and transpiration so that in some cases moisture was lowered below the intended minimum. When such sudden drying occurred, for example a pot in the 18 per cent. series which contained more than 19 per cent. of soil moisture at 10 A.M. contained only 15 per cent. soil moisture at 4:30 P.M., the leaves were limp and the scapes bent. The following day, after watering, the leaves were yellow and the scapes died more rapidly than expected. Such damage occurred to plants that were suddenly lowered below the previous low moisture condition except in the 6 per cent. series. Plants subjected periodically to severe drying seemed to become hardened and did not show signs of wilting even though they were sometimes subjected to as rapid drying as the plants on the more moist series. Soil temperatures varied with location on the bench but did not vary more than 1 degree within a replication.

After 24 weeks of growth, the number of rosettes was counted, and the tops were cut at the ground surface and weighed. The bulbs and tubers were washed free of soil and counted, and the tubers and rhizomes weighed. Dry weights were also determined, but there was no significant difference in the percentage of dry matter either for tubers, including bulbs, or tops, including scapes and umbels.

Results

The data were treated by analysis of variance and indicate that there was a highly significant difference between moisture levels in the number of shoots, the number of tubers, the weight of tops, the weight of tubers, and the tuber-top ratio, table I. The total weights of the plants and the dry weights of the tubers follow almost exactly the analysis for the weight of tubers, as would be expected from the similar percentages of dry matter and the fact that the tuber weight is from three to six times the top weight, and therefore dominates the total. The number of tubers for each rosette

above ground was extremely variable but no significant differences were shown.

TABLE I

VARIANCE* OR MEAN SQUARES FROM ANALYSES OF FIVE OBSERVATIONS ON EACH OF 45 POTS OF *Cyperus rotundus* L. GROWN WITH FIVE MOISTURE TREATMENTS IN THE GREENHOUSE AT TUCSON

SOURCE OF VARIANCE	DEGREES OF FREEDOM	NUMBERS OF TOPS	NUMBERS OF TUBERS	WEIGHT OF TOPS	WEIGHT OF TUBERS	TUBER/TOP RATIO
Moistures	4	1183.2†	5286.8†	1263.13†	17031.6†	7.39†
Replications	8	14.1	204.8	3.59	329.8	0.25
Interaction (error)	32	26.8	92.7	24.39	238.7	0.67

* An asterisk denotes a significant difference greater than the 0.05 per cent. level.
† A dagger denotes a significant difference greater than the 0.01 per cent. level.

TABLE II

AVERAGES AND DIFFERENCES BETWEEN DIFFERENT MOISTURE LEVELS MAINTAINED IN POTS OF *Cyperus rotundus* L. FOR NUMBERS OF TOPS AND TUBERS, WEIGHTS OF TOPS AND TUBERS, AND FOR THE TUBER/TOP RATIO

MINIMUM PERCENTAGE SOIL MOISTURE	NUMBER OF TOPS		NUMBER OF TUBERS		FRESH WEIGHT OF TOPS		FRESH WEIGHT OF TUBERS		TUBER/TOP RATIO	
	AVERAGE	DIFFERENCE	AVERAGE	DIFFERENCE	AVERAGE	DIFFERENCE	AVERAGE	DIFFERENCE	AVERAGE	DIFFERENCE
%										
6	8.7		28.3		4.1		26.3		6.4	
		4.7		17.4*		5.4*		24.1†		0.9*
9	13.4		45.7		9.5		50.4		5.5	
		5.9*		19.5*		7.0†		29.2†		0.6
12	19.3		65.2		16.5		79.6		4.9	
		8.0†		20.3*		8.4†		36.9†		0.1
15	27.3		85.5		24.9		116.5		4.8	
		10.2†		−3.8		8.8†		13.2		0.9*
18	37.5		81.7		33.7		129.7		3.9	
Significant differences at:										
0.05 per cent.	5.0		9.3		4.8		14.9		0.8	
0.01 per cent.	6.7		12.5		6.4		20.0		1.1	

Differences in excess of the 0.05 per cent. level of significance are marked with an asterisk (*), those in excess of the 1 per cent. level of significance are marked with a dagger (†).

Comparisons between adjacent moisture levels are shown in table II. The average number of tops per pot in the low moisture series was less than the number of tops in pots allowed to dry to only 9 per cent. soil moisture. The difference in number of tops between the two low moistures was not significant, but each further increase in the minimum moisture allowed was accompanied by a significant increase in the number of tops developed. The differences between the moisture series, mean of nine pots,

required to reach the five- and one-per cent. levels of significance are shown in the rows at the bottom of table II. The weight of the tops was similar to the number of tops but the difference in weight between the two low moisture levels was significant. The individual comparisons need not necessarily each be significantly different to show a significant over-all relation between the minimum soil moisture and the number and weight of tops. The very high probability of the significance of the relation is emphasized by the fact that every increase in the minimum moisture has increased top number and weight, and all but one of the individual comparisons was significant.

The number and weight of tubers developed was significantly different on the different moistures, as shown in table I. Comparisons of the individual averages show that the number of tubers failed to change significantly when the minimum moisture was increased above 15 per cent. Each increase in moisture from a minimum of six to a minimum of fifteen per cent. soil moisture resulted in a significant increase in both the number and weight of tubers.

The small difference in top weight between the two low moisture levels and the failure of the tuber weight to increase above 15 per cent. minimum moisture is shown most readily by the tuber-top ratio. A highly significant difference in tuber-top ratio on the different moisture levels is shown in table I. A more detailed study of the averages, table II, shows that the larger differences occurred between the two low and the two high moistures. The tuber-top ratio did not change significantly between 9 and 15 per cent. minimum moisture.

Discussion

Growth of plants in the field occurs with moisture fluctuating from saturation soon after a rain or irrigation to very dry soil or until another wetting which saturates the soil again. The natural differences in soil moisture are differences in volume of soil wet, and the frequency of wetting. In this experiment, the frequency of wetting was dependent upon the minimum soil moisture percentages permitted before the plants were irrigated. Some plants were not stressed for moisture at all, being always in soil wet above the moisture equivalent, and other plants were subjected to successively drier soil until the plants in the driest series were always subjected to soil dried below the wilting coefficient before being re-wet.

The plants were unable to grow in soil below the wilting coefficient, and were limited in total growth in the low series because of the time during which the soil was below this level. The difference in time available for growth could, therefore, account for the difference between the minimum moisture series of 6 and 9 per cent., but the plants in the 12-, 15-, and 18-per

cent. moisture series were never limited by complete lack of available moisture as measured by the wilting percentage, yet the plants responded to decreases of minimum moisture between 18 and 9 per cent. by decreased growth. The difference in growth through a range of soil moistures not usually considered limiting (**5**) may be the result of stoppage of food manufacture and transport, and particularly of growth (**4**) in the plant over the periods in the afternoons when transpiration had decreased the water content of the leaves. Significant differences in growth between the soil moisture levels would then be explained by the reduction in the time available for growth, as follows: Growth in the plants in soil containing 18 per cent. moisture would be interrupted only on the parts of warm days when the transpiration rate was high, while it would be restricted earlier in the afternoon and on more days in soil which was lowered to 15 per cent. moisture, and still longer interruptions would occur with each successive lowering of the minimum soil moisture, so that tuber development as well as top growth was checked on the 6-, 9-, and 12-per cent. soil moisture series.

The significant increase in the tuber-top ratio for plants grown on the lowest moisture level indicates that top development was limited by low moisture while the underground parts were still receiving sufficient food and moisture to provide some growth. The tuber-top ratio remained the same through the range of moisture from 9 to 15 per cent., indicating that, although total growth increased with increased moisture, the food supply was equally available, on the average, for both top and tuber development. The variations in the tuber-top ratio indicate that the moisture conditions affect the type of growth as well as the total quantity.

Summary

1. Each decrease in the minimum soil moisture percentage permitted before re-wetting to saturation resulted in a significant decrease in the weight of tops of *Cyperus rotundus* L.

2. Tuber development was decreased significantly by decreased soil moisture from a point 2 per cent. below the moisture equivalent.

3. Tuber-top ratios were significantly higher than average when the soil moisture minimum was below the wilting coefficient and significantly lower than average when the minimum soil moisture percentage was above the moisture equivalent.

4. Tuber growth was increased 150 per cent. and top growth 250 per cent. by raising the frequency of watering from a minimum of 9 to a minimum of 18 per cent. The 18 per cent. level of watering was nearly 100 per cent. better than the 12 per cent., even though both series were assumed to have a continuous supply of available moisture.

5. Under pot conditions, at least, growth may apparently be checked by

the availability of water in soil which was always wet above the wilting percentage.

DEPARTMENT OF AGRONOMY
THE UNIVERSITY OF ARIZONA

LITERATURE CITED

1. ALDRICH, W. W., WORK, R. A., and LEWIS, M. R. Pear root concentration in relation to soil moisture extraction in heavy clay soil. Jour. Agr. Res. **50**: 975–987. 1935.
2. DAVIS, C. H. Absorption of soil moisture by maize roots. Bot. Gaz. **101**: 791–805. 1940.
3. FURR, J. R., and TAYLOR, C. A. Growth of lemon fruits in relation to moisture content of the soil. U. S. Dept. Agr. Tech. Bull. 640. 1939.
4. LOOMIS, W. E. Daily growth in maize. Amer. Jour. Bot. **21**: 1–6. 1934.
5. VEIHMEYER, F. J. Some factors affecting the irrigation requirements of deciduous orchards. Hilgardia **2**: 125–291. 1927.

THE IRON–MANGANESE RATIO IN RELATION TO THE RESPIRATORY CO_2 AND DEFICIENCY–TOXICITY SYMPTOMS IN SOYBEANS

I. I. Somers, S. G. Gilbert, and J. W. Shive

(WITH ONE FIGURE)

The data for this brief report were obtained in the course of studies (**2**) dealing with the interrelationship between iron and manganese in plant growth and development. Since these metals are generally considered to function as catalysts in cellular respiration the relative proportions, both in the growth substrate and in the plant tissues, should be important in regulating the output of respiratory CO_2 from the roots of the plants. To determine the influence of the relation between iron and manganese upon the respiratory rate in soybean roots, measurements were made of the CO_2 production by roots of this species growing in substrates in which the relative proportions and concentrations of iron and manganese were varied between wide limits.

The details of the experimental procedure are given elsewhere (**1, 3**) and therefore, need no extensive discussion here. A special cultural technique permitted the measurement of the respiration of the roots of intact plants growing in solutions in which the oxygen tension was maintained at an approximately constant level during a prolonged experimental period. Seedlings, when ten days old, were transferred from a seed bed of pure quartz sand to a standard culture solution having the following composition: KH_2PO_4, 0.00058 M.; K_2SO_4, 0.0082 M.; $Ca(NO_3)_2$, 0.0045 M.; $MgSO_4$, 0.0022 M. The plants were grown for ten days in this solution before the experimental treatments were begun. The various iron-manganese treatments were continued for a period of ten days, at the conclusion of which the production of the respiratory CO_2 by the roots of the plants was measured over a 24-hour interval. The H-ion concentration of the culture solutions was maintained, both during the treatment period and during the interval of CO_2 measurements, at an approximately constant pH level of 4.5.

Iron in the form of $FeSO_4 \cdot 7H_2O$ and manganese in the form of $MnSO_4 \cdot 4H_2O$ were added to the culture solutions to provide a relatively wide range of concentrations of these two elements and an extreme range in the values of the ratio of iron to managanese from 0.001 to 1500. The concentration ranges in p.p.m. of iron and manganese added to the solutions, as well as the ratios of iron to manganese are given in table I, together with the average yield values of the root respiratory CO_2, in terms of millimoles per 10 grams of green plant tissue, corresponding to the respective ratios of iron to manganese.

From the data of table I, it will be observed that normal plants free from pathological symptoms were produced only when the ratio of iron to manganese in the substrate had values between 1.5 and 2.5. The highest yields of respiratory CO_2 also occurred within this range of ratio values. When the ratio Fe/Mn showed values either above or below this "optimal" range the plants invariably developed pathological symptoms which became progressively more severe with increase in the Fe/Mn ratio value above the optimal range and with decrease in the value of this ratio below the optimal range. Under these conditions the yields of respiratory CO_2 showed values very materially lower than those produced within the range of Fe/Mn

TABLE I

RELATION OF THE FE/MN RATIO TO THE PRODUCTION OF RESPIRATORY CO_2 BY ROOTS, AND PATHOLOGICAL SYMPTOMS IN TOPS OF SOYBEAN PLANTS GROWN IN CULTURE SOLUTIONS

Fe	Mn	Ratio Fe/Mn	pH of solutions	Average green weights	CO_2/10 gm. green weight	Pathological symptoms
p.p.m.	*p.p.m.*		*pH*	*gm.*	*millimoles*	
0.005	0.002	2.5	4.5	29.3	0.76	Normal plants
0.002	0.002	1.0	4.5	29.4	0.70	Slight Fe-deficiency (Mn-toxicity) symptoms
0.005	5.0	0.001	4.5	24.8	0.61	Very severe Fe-deficiency (Mn-toxicity) symptoms
0.5	0.002	250.0	4.5	26.8	0.57	Moderate Fe-toxicity (Mn-deficiency) symptoms
0.5	0.25	2.0	4.5	31.8	0.71	Normal plants
0.5	5.0	0.1	4.5	28.0	0.63	Moderate Fe-deficiency (Mn-toxicity) symptoms
3.0	0.002	1,500.0	4.5	26.2	0.54	Very severe Fe-toxicity (Mn-deficiency) symptoms
3.0	2.0	1.5	4.5	30.9	0.70	Normal plants
5.0	5.0	1.0	4.5	24.7	0.60	Slight Fe-deficiency (Mn-toxicity) symptoms

ratio values between 1.5 and 2.5. This very definitely associates low respiratory rates with an unfavorable balance between iron and manganese in the substrate and in the plant since the concentrations of active iron and active manganese in the plant tissues are determined by the concentrations of these elements in the substrate.

The pathological symptoms which developed in the plants under conditions of unfavorable balance between iron and manganese (Fe/Mn ratio values above 2.5 or below 1.5) and which are associated with low values of respiratory CO_2 yields are of two distinct types readily recognizable in the species here dealt with. At low values of the Fe/Mn ratio corresponding to low values of CO_2 yields, the pathological symptoms assume the characteristic chlorosis generally associated with iron deficiency and

are identical with manganese toxicity. Those which develop with high values of the Fe/Mn ratio corresponding to low values of CO_2 yields exhibit the characteristic necrotic condition associated with iron toxicity and are identical with manganese deficiency symptoms. Figure 1 presents a photograph of soybean leaves exhibiting these symptoms. These pathological symptoms develop with very low concentrations of both iron and manganese

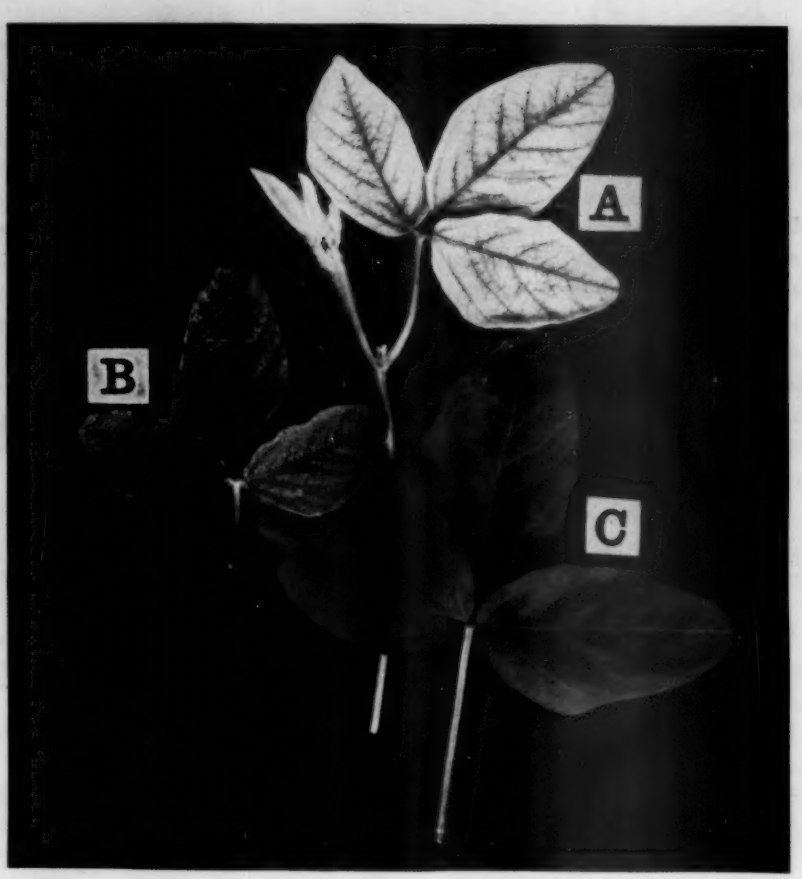


FIG. 1. Photograph of soybean leaves showing A, typical symptoms of Fe-deficiency (Mn-toxicity), and B, Fe-toxicity (Mn-deficiency). C, normal leaf.

or with excessively high concentrations of both these elements, if an unfavorable balance between the two elements occurs in the substrate or in the plants, as is clearly brought out by the data of table I.

Under the same experimental conditions normal plants with excellent vigor, high yields of plant material, high yields of respiratory CO_2 per

unit of plant material, and good color, can be produced with unbelievably low concentrations and with relatively very high concentrations of both iron and manganese provided that a favorable balance between iron and manganese exists in the substrate and in the plant. Thus, as is indicated in table I, excellent plants free from pathological symptoms were produced with iron concentrations of 0.005 p.p.m. and manganese concentrations of 0.002 p.p.m. (Fe/Mn ratio = 2.5); with iron and manganese concentrations 1000 times higher than these (Fe/Mn ratio = 1.5) plants were produced which were equally good in every respect. It is thus apparent that, within reasonable limits, the absolute concentrations of these elements either in the nutrient substrate or in the plant can be of no great importance so long as they are present in the proper relation one to the other.

The ratio of concentrations of these two trace elements has a profound influence upon the metabolism of the plant in general, since within the relatively short experimental period of ten days very marked pathological effects are produced by a deviation from the optimal range of the Fe/Mn concentration ratio either upward or downward. These effects are revealed by such quantitative aspects as growth, measured by weight production of plant material, as respiration, measured by the output of respiratory CO_2, as well as by the more qualitative aspects as manifested by the pathological developments usually described in terms of deficiency or toxicity of one or the other of these elements.

RUTGERS UNIVERSITY
NEW BRUNSWICK, NEW JERSEY

LITERATURE CITED

1. GILBERT, S. G. and SHIVE, J. W. The significance of oxygen in nutrient substrate for plants. I. The oxygen requirement. Soil Sci. **52**: 143–152. 1942.
2. SHIVE, J. W. Significant rôles of trace elements in the nutrition of plants. Plant Physiol. **16**: 435–445. 1941.
3. ————, and ROBBINS, W. R. Methods of growing plants in solution and sand culture. New Jersey Agr. Exp. Sta. Bull. 636. 1940.

BRIEF PAPERS

FAILURE OF ROOT TIPS OF TOMATO SEEDLINGS GERMINATED FROM X-RAYED SEEDS TO GROW IN VITRO

H. Kersten and G. F. Smith

(WITH ONE FIGURE)

Histological studies of roots of *Vicia faba* and of *Zea mays* seedlings grown from seeds given a medium dose of soft x-rays, indicate considerable distortion and irregularity about the apical meristem and surrounding tissues after the first week of germination (7, 8). These are not apparent during the early part of germination when the radicles first appear; the meristem is relatively normal in arrangement as compared with that in non-treated control seedlings (8).

In view of this effect, it was considered of interest to determine the ability of root tips of seedlings germinated from x-rayed seeds to continue growth *in vitro;* the results of which might indicate whether the abnormalities found at the apical meristem are localized effects of the x-ray radiation, or effects influenced more directly by functional irregularities occurring elsewhere in the root or in the seedling as a whole.

Unsoaked beef steak tomato seeds were irradiated 8 cm. from the focal spot of a copper target gas x-ray tube operated at 30 peak kv., and 10 ma. for 30 minutes. It was observed by separate tests that lateral roots do not appear during later germination of tomato seedlings from seeds given this x-ray treatment. The culture methods employed followed procedures listed by Bonner (4), Bonner and Addicott (5), Addicott (1), Addicott and Bonner (2), Addicott and Devirian (3), and Robbins and Bartley (6).

When root lengths of approximately 1 cm. were attained in aseptic seedlings from both irradiated and non-irradiated seeds, 5- to 10-mm. root tips (50 seedlings of each group) were cut and transferred to a previously sterilized nutrient medium containing: 236 mg. $Ca(NO_3)_2 \cdot 4H_2O$; 81 mg. KNO_3; 36 mg. $MgSO_4 \cdot 7H_2O$; 65 mg. KCl; 20 mg. KH_2PO_4; 1.5 mg. Fe-tartrate; 20 gm. sucrose, per liter of Pyrex redistilled water. Nicotinic acid (0.5 mg.), vitamin B_1 (0.1 mg.) and vitamin B_6 (0.1 mg.) were added per liter of nutrient.

The root tips of both seedling groups appeared to be alike in growth rate and size at the time of isolation. In view of the uniformity of the data, one repetition of the original results seemed adequate to confirm the observations made.

In figure 1A is shown the extent of growth of the excised root tips of control seedlings after four weekly transfers; B indicates that which occurred in the tissues excised from seedlings from the irradiated seeds. In the latter, the cultured root tips generally display only a very slight increase in length with some increase in thickness and, in addition, become inactive and browned at the apices soon after isolation.

FIG. 1. Shadow photographs of excised root tips of tomato. A, normal, at the end of four transfers; B, root tips of seedlings from x-rayed seeds, at the end of four transfers; and C, at the end of six transfers.

When transfers of tissues from the treated seeds were continued for five to six weeks, an activation of a few cells above the root tip frequently occurred, resulting in secondary root formation at these points (C). This occurrence might be attributed to a continued functioning of a few cellular elements having meristematic activity, which escaped effects induced by the x-ray radiation treatment of the seeds.

The inability of excised root tips of tomato seedlings grown from seeds given a medium dose of soft x-rays, to grow *in vitro* is probably associated

directly with factors active in continuous meristem activity in the root tip, present in the seed at the time of irradiation. The fact that the tissues did not grow in a nutrient medium adequate to support the continuous growth of normal root tips of tomato, seems to remove the alternative supposition that the effect is dependent upon modifications present in other parts of the germinating seedlings.

This work was repeated at the California Institute of Technology. Appreciation is expressed to Dr. F. W. WENT and to Dr. JAMES BONNER for comments and use of their laboratories.

DEPARTMENTS OF PHYSICS AND BOTANY,
UNIVERSITY OF CINCINNATI

LITERATURE CITED

1. ADDICOTT, F. T. Vitamin B_1 in relation to meristematic activity of isolated pea roots. Bot. Gaz. **100**: 836–843. 1939.
2. ————, and BONNER, J. Nicotinic acid and the growth of isolated pea roots. Amer. Jour. Bot. **26**: 661–665. 1939.
3. ————, and DEVIRIAN, P. S. A second growth factor for excised pea roots: nicotinic acid. Amer. Jour. Bot. **26**: 667–671. 1939.
4. BONNER, J. Growth of isolated pea roots. Amer. Jour. Bot. **27**: 692–701. 1940.
5. ————, and ADDICOTT, F. T. Cultivation *in vitro* of excised pea roots. Bot. Gaz. **99**: 144–170. 1937.
6. ROBBINS, W. J., and BARTLEY, MARY. Vitamin B_1 and the growth of excised tomato roots. Science n.s. **85**: 246–247. 1937.
7. SMITH, G. F., and KERSTEN, H. Root modifications induced in *Vicia faba* seedlings by irradiating unsoaked seeds with soft x-rays. Plant Physiol. **16**: 159–170. 1941.
8. ————, and ————. Root modifications induced in *Zea mays* seedlings by irradiating dry seeds with soft x-rays. Plant Physiol. **17**. 1942. (In press.)

STERILIZATION TECHNIQUE FOR GRASS SEEDS

RICHARD B. STEPHENSON

The seeds of lettuce, cantaloupe, and peas may be sterilized without physiological injury by being soaked for two hours in a saturated solution of Semesan (an organic mercury compound very slightly soluble in water, the

TABLE I

EFFECTS OF VARIOUS STERILIZATION PROCEDURES ON THE STERILIZATION AND GERMINATION OF KENTUCKY BLUEGRASS SEEDS

PRETREATMENT	STERILIZING AGENT	AFTER TREATMENT	GERMINATION	INFECTION
None	Saturated sol. Semesan for 30 min.	5% bromine water for 1 min.	None	None
None	1.5% sol. Semesan Bel for 30 min.	5% bromine water for 1 min.	None	None
None	10 mg. Ceresan dust per gm. seeds for 12 hr.	None	None	None
None	10 mg. Ceresan dust per gm. seeds for 12 hr.	Distilled water for 2 min.	None	Slight
None	0.1% sol. $Ca(OCl)_2$ for 15 min.	None	Good	Heavy
None	0.5% sol. $Ca(OCl)_2$ for 15 min.	None	Good	Heavy
None	1.0% sol. $Ca(OCl)_2$ for 15 min.	None	Good	Heavy
None	2.0% sol. $Ca(OCl)_2$ for 24 hr.	None	Fair	Moderate
95% alcohol for 1 min.	2.0% sol. $Ca(OCl)_2$ for 15 min.	None	Good	Moderate
95% alcohol for 1 min.	2.0% sol. $Ca(OCl)_2$ for 30 min.	None	Good	Very slight
95% alcohol for 1 min.	2.0% sol. $Ca(OCl)_2$ for 2 hr.	None	Good	None
95% alcohol for 1 min.	10.0% sol. $Ca(OCl)_2$ (filtered) for 30 min.	None	Good	None

active fraction of which is hydroxymercurichlorophenol) and then passed through a five per cent. solution of bromine water. The author has successfully cultured a great many seedlings and excised parts of seedlings in sterile nutrient media by this method of preliminary sterilization. The procedure, however, is completely lethal in its effect on the seeds of Kentucky Bluegrass. Semesan Bel, the active ingredients of which are hydroxymercurinitrophenol and hydroxymercurichlorophenol, and Ceresan, which contains ethyl mercury phosphate, both have mercury equivalents less than that of Semesan, yet likewise proved fatal to grass seeds. In an effort to obtain sterile grass seeds for subsequent germination and tissue culture, the procedures indicated in table I were tested.

The data in table I indicate that all compounds containing mercury were fatal to the seeds. Treatments with hypochlorite alone did not prevent normal germination, but they did not adequately sterilize the seeds. An adequate sterilization was obtained only when the seeds were pretreated with alcohol and then treated with hypochlorite. This is probably due to the fact that the surface tension effects of the alcohol caused thorough preliminary wetting of the seed coats after which the hypochlorite came into intimate contact with all surfaces. Many species of orchid seeds also are difficult to sterilize with hypochlorite unless they are previously thoroughly wetted with alcohol. The last treatment described in table I is the one routinely used by the writer.

Subsequent sterile germination of the grass seeds may best be carried out on two per cent. agar, rather than on sterile moist filter paper, because of the more constant supply of moisture to the seed and also because contamination is more easily observed.

UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

NOTES

Southern Section.—The Southern Section of the American Society of Plant Physiologists met with the Association of Southern Agricultural Workers at Memphis, Tennessee, on February 4 to 6, 1942. This was the third annual meeting, and the programs were interesting, the attendance good. Fifty or more participated in the fellowship, and had a very profitable experience. The retiring officers, Dr. LEWIS H. FLINT, Louisiana State University, chairman of the section, Dr. SAM F. THORNTON, of Norfolk, Virginia, and Dr. C. F. MORELAND, secretary-treasurer, Louisiana State University, Baton Rouge, Louisiana, are commended and congratulated for having provided an excellent program, and for the admirable arrangements for the comfort and convenience of the attendants. Two motion pictures were presented, one of which, *Potash production in the United States,* was presented by Dr. H. B. MANN of Atlanta, Georgia. This was timely because of the unavailability of foreign supplies of potash at the present time. The papers presented were mainly concerned with the problems of plant nutrition.

The Section seems to be well established now, and will take an ever increasing part in the development of scientific research in the Southern States. The officers elected for 1942–1943 are as follows: Chairman, Dr. S. F. THORNTON, F. S. Royster Guano Co., Norfolk, Virginia; Vice chairman, Dr. O. A. LEONARD, State College, Mississippi; Secretary-treasurer, Dr. I. E. MILES, Raleigh, North Carolina.

New England Section.—The New England Section of the American Society of Plant Physiologists will meet at Smith College, Northampton, Massachusetts, May 22–23, 1942. This central location, once called the Paradise of America by JENNY LIND, should attract an enthusiastic attendance. All plant scientists are invited. Men's colleges will probably be closed at this time. Teachers are invited to hold informal discussions on our services in the war effort. Address communications to Dr. DOROTHY DAY, chairman, the New England Section of the American Society of Plant Physiologists, Department of Botany, Smith College, Northampton, Massachusetts, or to the secretary, Dr. LINUS H. JONES, The Massachusetts State College, Amherst, Massachusetts.

Western Section.—The Western Section of the A.S.P.P. will hold its meeting with the Pacific Division of the A.A.A.S. presumably in Salt Lake City, Utah. These meetings usually occur about the middle of June. In the absence of more definite information, those who may desire to attend this meeting may obtain information by writing to Dr. J. VAN OVERBEEK, chair-

man of the section, at the California Institute of Technology, or to Dr. D. I. ARNON, secretary, at the Division of Plant Nutrition, The University of California. The Western Section meetings are always well attended, and a full program will be presented.

Program Committee.—The program committee for the New York meeting to be held in December, 1942, has been named by the President, Dr. EDWIN C. MILLER. The chairman of the committee is Dr. JOHN W. SHIVE, the New Jersey Agricultural Experiment Station, who has had much experience in handling meetings. Dr. W. O. DAVIDSON, of the same institution, will be his right hand man on the committee, and Dr. LINUS H. JONES, Massachusetts State College, is the third member. This will be our first meeting under the full impact of the war, and it is hoped that everyone will make an effort to attend, and to aid in all out service in any way that is open to us. The committee will need our sympathetic and cordial assistance. Let us make it a great meeting, not only in size, but in quality. Other announcements will be made in July and October if there is anything of importance to report.

Conservation of Scholary Journals.—The American Library Association has created, during the last year, a Committee on Aid to Libraries in War Areas, headed by JOHN R. RUSSELL. The Committee is faced with numerous problems, and hopes that American scholars and scientists will be of considerable aid in the solution of one of these problems.

One of the most difficult tasks in library reconstruction after the first World War was that of completing foreign institutional sets of American scholarly, scientific, and technical journals. The attempt to avoid a duplication of that situation is now the concern of the Committee.

Many sets of journals will be broken by the financial inability of the institutions to renew subscriptions. As far as possible they will be completed from a stock of periodicals being purchased by the Committee. Many more will have been broken through mail difficulties and loss of shipments, while still other sets will have disappeared in the destruction of libraries. The size of the eventual demand is impossible to estimate, but requests received by the Committee already give evidence that it will be enormous.

With an imminent paper shortage attempts are being made to collect old periodicals for pulp. Fearing this possible reduction in the already limited supply of scholarly and scientific journals, the Committee hopes to enlist the cooperation of subscribers to this journal in preventing the sacrifice of this type of material to the pulp demand. It is scarcely necessary to mention the appreciation of foreign institutions and scholars for this activity.

Questions concerning the project or concerning the value of particular

periodicals to the project should be directed to WAYNE M. HARTWELL, Executive Assistant to the Committee on Aid to Libraries in War Areas, Rush Rhees Library, University of Rochester, Rochester, New York.

Preparation of Manuscripts.—Many of the papers submitted to PLANT PHYSIOLOGY for publication are in a poor state of preparation, or are in such form that they require hours of work and retyping to make them fit copy for the printer. Much of the difficulty is the result of inexperience, ignorance of our needs, thoughtlessness, or lack of imagination. The fact that some authors turn in almost flawless copy is evidence that it is not necessary to send carelessly prepared and slovenly manuscripts. Believing that many authors would strive to improve their manuscripts if given brief directions from the editors' slant, the following suggestions are offered:

1. Examine every feature of the journal you are asking to use your manuscript, and try to conform to its practices. Note the position of headings, general organization, and the methods of citing literature particularly, and bring your paper into conformity with the general architecture of papers published.

2. Do not crowd the material. Leave a 3-inch margin at the top of the first page, liberal side margins for editorial marks, and double spacing of lines so that there is space for any required editorial changes.

3. Send clean copy. If you find penciling necessary after the copy has been prepared, retype it and submit without penciling or pen corrections.

4. Avoid footnotes as far as possible. If you do use them, number them with Arabic numbers. They are expensive, can usually be incorporated in the text, and detract from the appearance of the printed page.

5. Identify your figures. Place your name, and figure number on the back of each figure. Do not put legends into or on the figure, either front or back. Type all legends, properly numbered to correspond to the figures, on a sheet of your manuscript.

6. Do not edit the paper before sending it. Leave headings unscored, scientific names without underlining, etc. The editors will take care of all of these matters in accordance with the practice of the journal. Generic names, used alone, are not italicized.

7. Place each table on a separate page; do not crowd too much material into a single table. Note size of page (width and length, $4\frac{1}{2} \times 7$ in.) and gauge tables accordingly, leaving plenty of space in margins and in the interior for editorial marks. Use horizontal lines at top and bottom, not in interior of tables, and vertical lines between columns. Spaces (leads) are used to separate sets of data. For footnotes to tables, use the asterisk, dagger, double dagger, and section. Tabular material should be used sparingly. It costs over $10.00 per page.

8. Acknowledgments are preferred at the end of the paper. The name of your institution and its address should be given at the end of the manuscript.

9. Use separate pages for the literature cited. Give complete citations, author, title, journal name, vol. no., inclusive pages, and year. (See any literature list in the journal for examples.) Punctuate according to the examples, and leave no citations incomplete.

10. Limit the size of all pages, drawings, tables, etc., to standard $8\frac{1}{2} \times 11$ inches, or less.

In general, use typewriter ribbons that are not too dry; send originals and retain a carbon copy to insure against loss, and to consult in case of need. Use mathematical formulae only when necessary, and be conservative in the use of space. We solicit your cooperation in this effort to relieve the editors and printers of some of their most serious and trying problems.

Early Volumes Wanted.—There are occasional requests from libraries for the early volumes of PLANT PHYSIOLOGY. The Business Manager, Dr. J. FISHER STANFIELD, Chicago Teachers College, Chicago, Illinois, will be pleased to hear from any members who desire to part with complete volumes 1 to 4 inclusive, or any one complete volume in this series. You can obtain more for these volumes than they cost you, as they now are at a premium. If you wish to sell, send in your lowest cash offer. The bidding price is $7.50 per volume.

Colloid Science.—The Interscience Publishers, Inc., 215 Fourth Ave., New York, have initiated a series of scientific works which present the advances being made in special fields of research. One of these series is entitled *Advances in Colloid Science*. The editors for the first volume of the series are E. O. KRAMER, F. E. BARTELL, and S. S. KISTLER. The text, of 434 pages, consists of twelve papers, or reviews, with titles as follows: The measurement of the surface areas of finely divided or porous solids by low temperature isotherms, by P. H. EMMETT; The permeability method for determining specific surface of fibers and powders, by R. R. SULLIVAN and K. L. HERTEL; A new method of adsorption analysis and some of its applications, by A. TISELIUS; Solubilization and other factors in detergent action, by J. W. McBAIN; Recent developments in starch chemistry, by K. H. MEYER; Frictional and thermodynamic properties of large molecules, by R. E. POWELL and H. EYRING; The constitution of inorganic gels, by H. B. WEISER and W. O. MILLIGAN; The creaming of rubber latex, by G. E. VAN GILS and G. M. KRAAY; Streaming birefringence and its relation to particle size and shape, by J. T. EDSALL; Synthetic-resin ion exchangers, by R. J. MYERS; The study of colloids with the electron microscope, by T. F. ANDERSON; and Anomalies in surface tensions of solutions, by E. A. HAUSER.

It is remarkable that one of these papers comes from Sweden, one from Switzerland, and one from Java. The remainder are of American authorship. Each review has its own literature list, some over a hundred each, and the author and subject indexes make the volume very easily used. There are 161 illustrations.

The reviews are well done, clear, readable, up to date, and accurate. The press work has been very well done. It is a pleasure to commend the series

to the serious attention of all physiologists; for the colloidal state is fundamental, and must be understood before physiological behavior may be understood. The price of this excellent work is $5.50 per copy, and the orders should be addressed to the publishers.

Advances in Enzymology.—A second volume of *Advances in Enzymology* deals with the following topics: Bacterial viruses (bacteriophages), by M. DELBRUCK; The kinetics of hydrolytic enzymes and their bearing on measuring enzyme activity, by D. D. VAN SLYKE; A classification of proteolytic enzymes, by M. BERGMANN; The enzymic properties of peptidases, by M. J. JOHNSON; Diamin-oxydase, by E. A. ZELLER; The chemistry of tea-fermentation, by E. A. H. ROBERTS; Heterotrophic assimilation of carbon dioxide, by C. H. WERKMAN and H. G. WOOD; Atmung, Gärung und die sich daran beteiligenden Enzyme von Aspergillus, by H. TOMIYA; Cellulose decomposition by microorganisms, by A. G. NORMAN and W. H. FULLER; A unified hypothesis of the reciprocal integration of carbohydrate and fat catabolism, by E. J. WITZEMANN; Vitamin K, its chemistry and physiology, by H. DAM; and The adrenal cortical hormones, by J. J. PFIFFNER.

The editors of this admirable volume are F. F. NORD, and C. H. WERKMAN. TAMIYA's paper is printed in German. Literature citations are copiously supplied, and author and subject indexes make reference reading easy. As this is the second volume on enzymology, there is a brief cumulative index to both volumes. There are 23 illustrations.

The quality of the reviews is good, and one must commend the issuing of these helpful works when investigations are numerous, widely scattered, and beyond any but the better libraries. No one can keep up with the currents of research without such monographic reviews.

This is another Interscience Publishers, Inc. book, priced at $5.50 per copy. Orders should be sent to the company at 215 Fourth Ave., New York.

Soils and Fertilizers.—Students have been familiar with Dr. FIRMAN E. BEAR's excellent book on *Soil Management* for many years. The third edition, just issued by John Wiley and Sons, has been changed in title to read *Soils and Fertilizers*, because of the ever increasing importance of mineral additions to the soil for maintenance or improvement of soil fertility. There are twenty-six chapters, with titles slightly changed from earlier editions, but covering the same topics, in the same logical order. It is simply and clearly written, and is a very good elementary text-book in its field. Especially adapted to beginners in the study of soil science. The price of the book is $3.50 per copy.

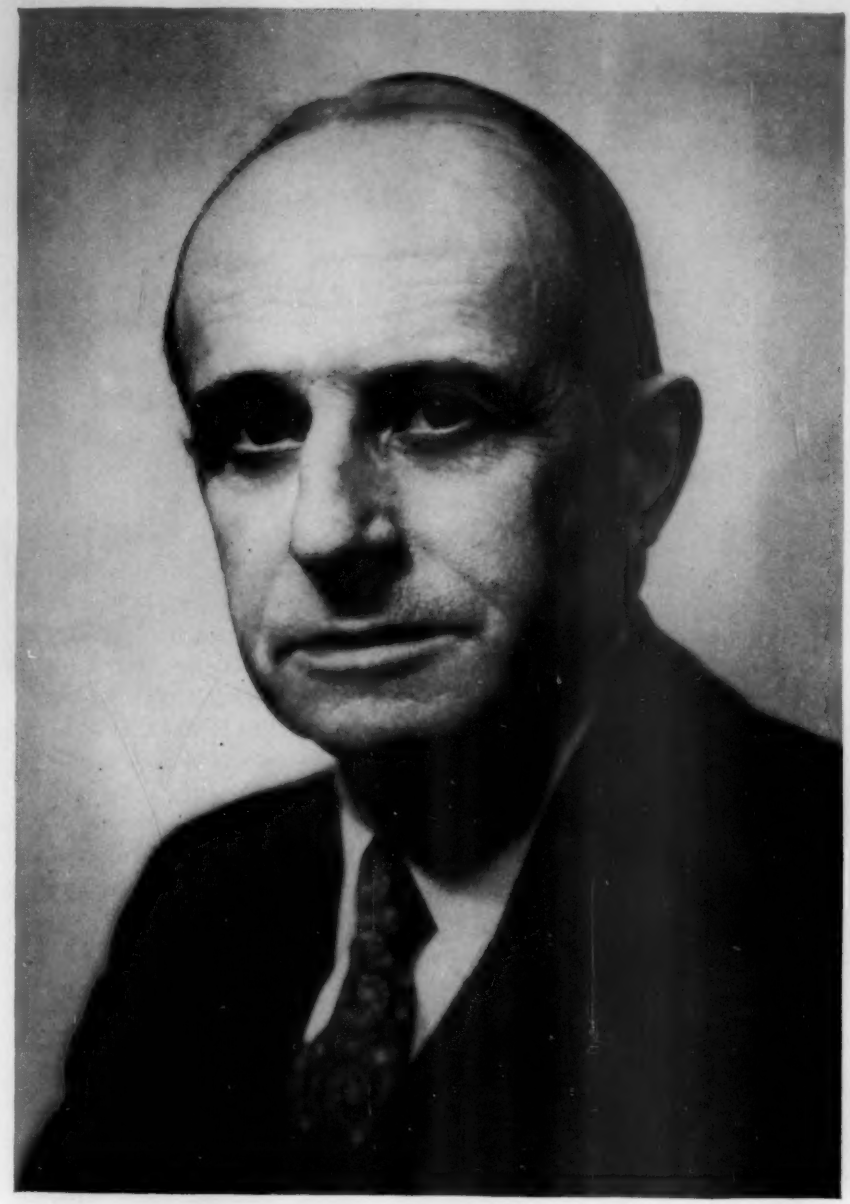
THIS NUMBER OF PLANT PHYSIOLOGY

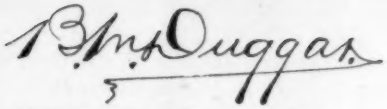
IS DEDICATED TO

BENJAMIN MINGE DUGGAR

THE SEVENTIETH ANNIVERSARY OF HIS BIRTH

SEPTEMBER 1, 1942





BENJAMIN MINGE DUGGAR
SEPTEMBER 1, 1872